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RODENT BIOLOGY AND INTEGRATED PEST MANAGEMENT IN AFRICA

Proceedings of the International Workshop held in Morogoro

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PREFACE

Rodents have been a problem to mankind since many thousands of years. Crop destroyers and carriers of diseases, rats and mice have stimulated man's endeavours to find ways of killing the vermin. Yet, rodents are still out there and thriving as well as ever. They pose continuing pest problems even in the richer parts of the world where elaborate prevention and control programmes have been set up. In developing countries, where economic constraints often prevent adequate pest management, the situation is much worse. Agricultural production is barely sufficient for survival in many of these countries and any pest damage may tip the balance to shortage or even famine. Hygienic standards are generally lower and public health systems less well equipped, so that diseases transmitted by rodents may spread faster and cause an increased morbidity.

Africa has more than its share of rodent problems with a variety of species that attack different crops and carry many diseases. Rodent damage on fields and in stores is an annual phenomenon but several species show irregular population explosions and damage figures of up to 80 % have been reported. Two classic examples of rodent zoonoses are prominently present: plague is endemic in Madagascar, Tanzania and the neighbouring countries; Lassa fever is an important health problem in West Africa. FIEDLER (1988) reviewed rodent problems in Africa in detail[1]. In his discussion, he attributed the failure of rodent control on that continent to poor biological knowledge about the concerned species and the lack of trained and well-equipped rodent scientists.

With this background in mind, we started a collaborative project in 1994 with scientists from Tanzania, Ethiopia, Norway, Denmark and Belgium. The objective of this project was to investigate several aspects of rodent biology in East Africa and integrate such knowledge for pest management; financial support was provided mainly by the Commission of the European Union (DG XII). Knowing that several other groups of researchers were addressing similar issues, we organised the «International Workshop on Rodent Biology and Integrated Pest Management in Africa» in Morogoro, Tanzania, from 21-25 October 1996. Morogoro, about 200 km west of Dar es Salaam, was an obvious place for this Workshop; the Rodent Control Centre of the Ministry of Agriculture and the Rodent Research Unit at the Sokoine University of Agriculture in this town have been active in rodent control and research during the past 15 years (with assistance from Denmark, Belgium, Canada, Sweden, Germany and the European Union) and still continue to do so. There were 62 registered participants at the Workshop, coming from 26 coun-

[1] FIEDLER, L.A. (1988) – Rodent problems in Africa. In: *Rodent pest management*. PRAKASH, I. (Ed.). CRC Press, Inc., Boca Raton: 35-65.

tries of which 16 in Africa. There were 50 oral presentations and 13 posters, divided in sessions on taxonomy, population dynamics, behaviour, physiology, zoonoses epidemiology and integrated pest management. A mini-symposium on plague epidemiology in Tanzania was convened by B.S. Kilonzo and incorporated in the Workshop program.

The aim of the Workshop was not so much to discuss rodent problems and control techniques in Africa, but rather to bring together rodent scientists with different experiences and see how their knowledge could contribute to improved rodent management strategies in Africa. This issue of the *Belgian Journal of Zoology* contains the proceedings for the Workshop. Many of the presented studies at the Workshop were in initial phases, not yet allowing publication; others were ready to be published elsewhere. Although the present proceedings thus do not contain contributions from each participant, they provide a very good overview of the different topics discussed in Morogoro. The papers published here also support the Workshop recommendations for future strategic and applied research which were formulated at the end of the meeting. These recommendations can be summarized as follows:

– The taxonomy of African pest rodents, and their parasites, still needs a lot of multidisciplinary study. Ecologists and rodent control specialists should be stimulated to collect material for taxonomical purposes. Taxonomists should design a standardized collection protocol and a coordinated distribution and communication system. The same material could also be made available for research on the epidemiology of zoonotic infections. Reference collections are needed both locally and in international museums.

– Ecological studies should attempt to collect much longer time series and analyse these properly. Too many of the short-term studies are still purely descriptive and basically replicates of earlier work; such studies would benefit from an intensified experimental approach. Ecological modelling of African rodent populations, temporally and spatially, will be useful in improving our understanding of the dynamics but also in forecasting rodent problems and simulating management strategies.

– The differences in life-history characteristics between closely related rodent pest species in Africa call for comparative studies of the physiology of these rodents.

– Increased activities in the field of medical ecology are desirable. The epidemiological patterns of rodent-borne zoonoses like plague and many others are still not clear. Community-ecology studies are urgently needed to understand the interaction between rodents, their parasites and pathogens and the environment.

– The study of plague epidemiology is hampered by the poor diagnostic services that are available locally. Improvement of these facilities and the used techniques, and an organised reference system to an African research center are required.

– Rodent control still follows the principles of Integrated Pest Management (IPM) only rarely. A more selective use of rodenticides requires increased research efforts on forecasting, repelling and prevention, and on the sustainability of rodent IPM strategies. Damage assessment techniques must be adapted and refined to evaluate control approaches. Simultaneous control of rodents and their ectorparasites should be developed for areas with enzootic arthropod-transmitted infections. There is a need for high-level education of rodent control scientists.

– Rodent control should be integrated in the existing IPM systems. Newly developed knowledge should be transmitted more effectively to private end-users; their participation in rodent management should be stimulated. There is much to learn from the experience that has been gained in Asia in this respect.

– The knowledge on population dynamics, epidemiology of rodent-transmitted zoonoses and management of rodents is fragmented and scattered. A central database about this knowledge and the relevant expertise should be established, if possible, under auspices of international organizations like FAO/WHO.

The Workshop Organizing Committee is confident that the Morogoro meeting has succeeded in its prime goal of bringing African rodent scientists together and confront their ideas with the needs and facts of rodent control. We hope that the above recommendations may stimulate further investigations in African rodent biology and that these studies may contribute to improved management strategies for rodent problems on the African continent.

ACKNOWLEDGEMENTS

The success of the Workshop would not have been possible without the extensive financial support from the European Commission (DG XII - B4). Due to their generous help, we could contribute to the travel and participation costs of 23 participants and publish these proceedings. On behalf of the whole Organizing Committee, I would like to extend special thanks to Tim Hall for his encouragement.

It was a great experience to organize this Workshop with Robert Machang'u and Patrick Mwanjabe, the two members of the Local Organizing Committee. We had an incredibly hectic period, but it was worth it! The Sokoine University of Agriculture, Morogoro, hosted the Workshop and provided facilities and staff to ensure a perfect international meeting. The Rodent Research Unit at this University and the Rodent Control Centre of the Ministry of Agriculture in Morogoro took care of practical and administrative preparations. All travel grants were successfully administered by N.Wouters at the University of Antwerp (RUCA); staff and students of the Evolutionary Biology group there, and the University financial administration, are thanked for their support. Walter Verheyen, co-ordinator of the project from which the Workshop originated, guided me through all initial stages of the organisation.

Much appreciation also goes to the referees who commented the manuscripts for these proceedings: N. Bille, G. Bronner, C. Chimimba, F. Clark, M. Corti, M. Desmecht, J.M. Duplantier, A. El-Sherbiny, S. Feresu, L. Fiedler, K. Gage, L. Granjon, N. Gratz, A. Hailu, D. Happold, H. Henttonnen, A. Krogfelt, K. Larsen, J. Lodal, R. Mathur, N. Oguge, H. Posamentier, E. Schockaert, G. Singleton, R. Smith, J. Stuyck, A. Vaheri, E. Van der Straeten, R. Verhagen and N. Watson. A. Rasmussen improved the language in several papers. I am also much indebted to Ernest Schockaert, the Editor of the *Belgian Journal of Zoology*: without his help, these proceedings would not have looked nearly as nice as they do. Finally, I want to thank Ann, for bearing with my travels abroad and my regular virtual absence even when at home.

HERWIG LEIRS
(Lyngby, 21 July 1997)

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SYSTEMATICS OF THE GENUS *MASTOMYS* (Thomas, 1915) (Rodentia: Muridae)

A REVIEW

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Key words: Mastomys, systematics, chromosomes, species limits.

Abstract. An update of the systematics of the genus *Mastomys* is presented, based on a bibliographical analysis and recent results obtained in various fields. Seven species are considered, namely *M. erythroleucus, M. coucha, M. shortridgei, M. natalensis, M. huberti, M. pernanus* and *M. verheyeni. M. hildebrandtii*, listed by some authors, is considered here as *species inquirenda*, due to insufficient evidence. The main characteristics of these species are described, with special emphasis on the karyotype, which appears to be an especially informative, species-specific character in the genus. The known distribution of each species is mapped, and the various intrageneric phylogenetic hypotheses are presented. The difficulties that remain in this group are listed, together with some directions in which further research should be carried on.

Key words : Mastomys, systematics, chromosomes, species limits.

INTRODUCTION

Distributed throughout Africa south of the Sahara with a relict population in Morocco, the genus *Mastomys* Thomas, 1915 undoubtedly represents one of the major components of the African mammal fauna from various points of view. Except in the primary forest region where it is restricted to human settlements, it is often one of the dominant genera of the small mammal communities. At least two of its species, *M. erythroleucus* and *M. natalensis*, regularly display spectacular population explosions, making them important pests for standing crops and stored foods (POULET, 1982; LEIRS, 1994). Also, some species of the genus are known to be reservoirs for various infectious diseases affecting humans, including bubonic plague and Lassa fever (see LEIRS, 1994).

All these characteristics have made *Mastomys* probably the most studied rodent taxon among the indigenous African murids. Nevertheless, it is only recently that its taxonomy

has become clearer, and that it has been nearly unanimously recognized as deserving a genus rank. This status was already admitted by ALLEN (1939), after THOMAS (1915) has proposed the taxon *Mastomys*, together with *Praomys* and *Myomys*, as a subgenus of *Epimys* Trouessart, 1881. ELLERMAN (1941) considered all these taxa as subgenera of *Rattus* L., 1758, while MISONNE (1971) placed them within the genus *Praomys*, followed by HONACKI *et al.* (1982). In the meantime, ROSEVEAR (1969) discussed the situation of *Mastomys* as a separate genus, as later did MEESTER *et al.* (1986), and MUSSER & CARLETON (1993). First suggested on morphological grounds, the monophyly of *Mastomys* was subsequently demonstrated via chromosomal analysis (MATTHEY, 1958; LEE & MARTIN, 1980; BRITTON-DAVIDIAN *et al.*, 1995), multivariate analyses of biometrical data (VAN DER STRAETEN, 1979; VAN DER STRAETEN & ROBBINS, 1997) and molecular results (CHEVRET *et al.*, 1994).

Species definition and characterization have also greatly improved since ELLERMAN (1941) who listed 28 forms (except for *M. pernanus)* as subspecies of *Rattus* (*Mastomys*) *coucha*. Here, the use of genetic techniques (*sensu lato*, and especially protein electrophoresis and chromosome analysis), has been of paramount importance in discriminating sibling species (MEESTER, 1988). GREEN *et al.* (1980), HUBERT *et al.* (1983), ROBBINS & VAN DER STRAETEN (1989), LAVRENCHENKO *et al.* (1992), MUSSER & CARLETON (1993) and BRITTON-DAVIDIAN *et al.* (1995) have synthesized the available data and made various propositions regarding the intrageneric taxonomy of *Mastomys*. We here update these data, taking into account the most recent findings that have shed additional light on the systematics of the genus. For the species list, we basically follow MUSSER & CARLETON (1993), with two exceptions:

First, we do not find sufficient data to support *M. hildebrandtii* (Peters, 1878) as presented in MUSSER & CARLETON (1993). QUMSIYEH *et al.* (1990) considered the 2n=32, FN=50-54 *Mastomys* specimens from Kenya as belonging to the same species as those with similar karyotypes found in Somalia by CAPANNA *et al.* (1982) who called them *M. huberti.* But QUMSIYEH *et al.* (1990) then synonymized *M. huberti* and *M. hildebrandtii*, giving priority to the older name *M. hildebrandtii*. MUSSER & CARLETON (1993) followed QUMSIYEH *et al.* (1990), but added that *M. huberti* is the species with 2n=32, and an autosomal fundamental number aFN=44, thus implicitely recognizing a synonymy between two entities with very different fundamental numbers aFN=50-54 and aFN=44, which is very doubtful from a chromosomal point of view. Moreover, this 2n=32, FN=44 karyotype (actually aFN=44-46, see below) has in fact never been found in East Africa, where the type specimen of *M. hildebrandtii* comes from (Kenya). Rather, this karyotype is restricted to West Africa, where it may well correspond to *M. huberti* described from northern Nigeria. That is why we here list *M. huberti* as a more likely valid species, following PETTER (1977), and awaiting comparative studies including karyotyped specimens, the holotype and series from the type locality of *M. huberti.* The existence of an East African species distinct from *M. natalensis*, and that may be *M. hildebrandtii,* is not sufficiently supported to date (see discussion), hence we prefer to consider it as *species inquirenda.*

Second, we do not include the species *angolensis* in the taxon *Mastomys*, considering as an important character of the genus the number of mammae of the females, following ELLERMAN (1941, p.168: «mammae usually more than 12, not separated into pectoral and

inguinal sets»). In that, we disagree with CRAWFORD-CABRAL (1989), who discarded the mammae count (3-2=10) as a significant character and maintained *angolensis* within *Mastomys* (see ROBBINS & VAN DER STRAETEN (1989), and MUSSER & CARLETON (1993) for further details). We will first characterize as completely as possible the species of the genus, briefly discuss their phylogenetic relationships, then state the problems that remain to be solved, and the subsequent directions of research that we consider as the most important.

SPECIES CHARACTERIZATION

Mastomys erythroleucus (Temminck 1853)

The species was described from Ghana on the basis of a young specimen of unknown sex, the skull of which was in poor condition (ROBBINS & VAN DER STRAETEN, 1989). ROBBINS & VAN DER STRAETEN (1989) questioned the validity of *M. erythroleucus* for animals having a diploid number of 38 chromosomes, a correspondence proposed by PETTER (1957; 1977). We support the now widely admitted view (DUPLANTIER *et al.*, 1990a) that *M. erythroleucus* is a valid species: the name *erythroleucus* refers to a fur color pattern which indeed characterizes individuals with 38 chromosomes. The biometric variability between 2n=38 populations that ROBBINS & VAN DER STRAETEN (1989) mention, is part of the more general problem of intraspecific polymorphism that has been illustrated in *Mastomys* species by various authors (DUPLANTIER, 1988; DIPPENAAR *et al.*, 1993; GRANJON *et al.*, 1996).

The first mention of this karyotype was by MATTHEY (1958, corrected in 1965 and 1966a), based on a specimen from Ivory Coast. The common range of autosomal fundamental number (aFN) recorded so far for this species is 50-56 (DUPLANTIER *et al.*, 1990a). Belonging to this chromosomal form are specimens from Morocco (TRANIER, 1974), Senegal (HUBERT *et al.*, 1983; DUPLANTIER *et al.*, 1990a; BRITTON-DAVIDIAN *et al.*, 1995), Burkina Faso, Mali and Niger (SICARD, pers. comm.; BRITTON-DAVIDIAN *et al.*, unpubl. data), Ivory Coast (MATTHEY, 1958; 1965; 1966a), Benin (CODJIA *et al.*, 1996), Cameroon and East Zaïre (MATTHEY, 1967), and Ethiopia (ORLOV *et al.*, 1989; BASKEVICH & ORLOV, 1993). Specimens with 38 chromosomes have also been found in other localities, but either the aFN was not reported: Sierra Leone and Cameroon (ROBBINS & VAN DER STRAETEN, 1989), Nigeria (DOBROKHOTOV, 1982), Burundi (VERHEYEN, pers. comm. in ROBBINS *et al.*, 1983), or the aFN was outside the range defined above: Central African Republic (aFN=68 or 70, MATTHEY in HUBERT *et al.*, 1983), East Zaïre (aFN=60; KRAL, 1971), Sudan (aFN=40; VIEGAS-PEQUIGNOT *et al.*, 1987).

Protein electrophoresis has been performed in a number of studies, mainly on hemoglobin, in order to distinguish between sympatric chromosomally differentiated species of *Mastomys*. Such instances where the 2n=38 *Mastomys* analyzed are likely to be *M. erythroleucus* specimens, include the studies of DOBROKHOTOV (1982) in Nigeria, and ROBBINS *et al.* (1983) in Sierra Leone. As stated above, the aFN of these 38-chromosome *Mastomys* individuals was not reported in either cases, nor were those of the 32-chromosome individuals to which they were compared (which could have been either *M. natalensis* or *M. huberti*). It is worth noting, however, that in both studies, the two chromosomal forms were distinguished on the basis of their hemoglobin pattern, and that an impor-

tant variability was observed within the 38-chromosome form (see LAVRENCHENKO *et al.*, 1992). In a more thorough allozymic analysis, DUPLANTIER *et al.* (1990b) studied a sample of *M. erythroleucus* from Senegal at 20 loci and found not only low levels of interpopulation divergence (a conclusion already suggested by KAMINSKI *et al.*, 1987), but a very slight overall differentiation from *M. huberti* (D_{Nei}=0.118) with no fixed allelic differences between the two species.

From a biometrical and morphological point of view, no single body or skull measurement has been found to unambiguously discriminate between *M. erythroleucus* and *M. huberti* and *M. natalensis* in Senegal (DUPLANTIER, 1988). The same is true for dental patterns and measurements (DENYS *et al.*, unpubl. data). However, the use of multivariate discriminant analyses on cranial and mandibular (DUPLANTIER, 1988) or dental (DENYS *et al.*, unpubl. data) measurements has achieved a nearly complete separation of these three species, at least for specimens from Senegal. Characters that are being more and more used as taxonomic tools are sperm and penis morphology and dimensions. They appear very useful for differentiating *M. erythroleucus* from other species of the genus (BASKEVICH & LAVRENCHENKO, 1995; LAVRENCHENKO & BASKEVICH, 1996).

M. coucha (Smith, 1836)

The species was described from Kuruman, South Africa, and since the synthesis of GREEN *et al.* (1980) who proposed to use this name only for the 2n=36 *Mastomys* from Southern Africa, there has been a general consensus on this point. MATTHEY established the karyotype as early as 1954, and further commented on it in 1958 and 1966(a), reporting the aFN as ranging between 52 and 54. The G-banded pattern is described for specimens from Zimbabwe (LYONS *et al.*, 1977) and South Africa (LEE & MARTIN, 1980) where HALLETT (1979) also mentioned some variability (aFN=54-56). *M. coucha* is only known from Southern Africa, being recorded from South Africa, Zimbabwe and Namibia (see map in SKINNER & SMITHERS, 1990). The individual from Central African Republic with a 2n=36, aFN=56 karyotype described by MATTHEY (1970) may represent another species or an aberrant specimen.

Associated with this karyotype, a specific electrophoretic pattern of hemoglobin («fast») was found (GORDON, 1978; GREEN *et al.*, 1980), consistently different from that of the 32-chromosome *M. natalensis*. Here again, only multivariate discriminant analyses on skull measurements efficiently separated *M. coucha* from *M. natalensis* (DIPPENAAR *et al.*, 1993; NJOBE, unpubl. data). Sperm morphology which appeared very similar between these two species (BREED, 1995), nevertheless allowed *M. coucha* to be distinguished from *M. shortridgei,* a species with a very similar karyotype (GORDON, 1985).

M. shortridgei (St Leger, 1933)

Little has been published on this species which is considered to be restricted to the extreme NE of Namibia and NW of Botswana (see map in SKINNER & SMITHERS, 1990). GORDON (1985) described its mammary formula as being 8:8=16, whereas SKINNER & SMITHERS (1990), following older descriptions, mentioned only 5 pairs of mammae (as in the type-specimen, where the nipples lie in one line, and are not grouped). The karyotype

is very similar to that of *M. coucha*, with 2n=36, aFN=50 with an almost complete G-band homology, although the sex chromosomes were found to differ using C-banding. Sperm head shape was also distinctive between these two species (GORDON, 1985).

M. natalensis (Smith, 1834)

The species was described from Durban (South Africa), and as argued by GREEN *et al.* (1980), *M. natalensis* undoubtly represents the valid species name for the 32-chromosome *Mastomys* individuals from Southern Africa. In this region, this diploid number seems to correspond to only one species. The situation is more complicated in other parts of Africa (DUPLANTIER *et al.*, 1990a; BASKEVICH & ORLOV, 1993), where at least two species with 2n=32 are present (*M. natalensis* and *M. huberti* in West Africa, *M. natalensis* and *Mastomys* sp. in East Africa, see discussion). For this reason, DUPLANTIER et al. (1990a) suggested that *M. natalensis* be described by the combination of both its 2n (=32) and aFN (=52-54).

This karyotype was first found by MATTHEY (1955) in Ivory Coast, then in Central African Republic, Congo (MATTHEY, 1966a) and Chad (MATTHEY, 1966b). In these regions, the Y chromosome was described as submetacentric whereas it was scored as an acrocentric chromosome in specimens from all other areas. In West Africa, *M. natalensis* is also known from Senegal (DUPLANTIER et al., 1990a), Benin (CODJIA *et al.*, 1996), Burkina Faso, Mali and Niger (HUBERT *et al.*, 1983; SICARD, pers. comm.; BRITTON-DAVIDIAN *et al.*, unpubl. data). The same karyotype was described from Somalia (CAPANNA *et al.*, 1982), Ethiopia (ORLOV *et al.*, 1989; BASKEVICH & ORLOV, 1993) and Tanzania (LEIRS, 1994). HUBERT *et al.* (1983), citing DOBROKHOTOV *et al.*, mentioned this karyotype in Nigeria, although DOBROKHOTOV (1982) did not report the aFN of the 32-chromosome *Mastomys* individuals he studied in this country. In Southern Africa, the presence of *M. natalensis* was confirmed in South Africa and Namibia (HALLETT, 1979) and Zimbabwe (LYONS *et al.*, 1980). Finally, 32-chromosome *Mastomys* individuals were mentioned from Sierra Leone and Burundi (ROBBINS *et al.*, 1983), but the aFN was not reported. The conspecificity of *M. natalensis* from Senegal and South Africa (i.e. the two extremes of the species range) has recently been definitely proven by GRANJON *et al.* (1996), which probably makes this species the most widely distributed mammal of Africa.

Protein electrophoresis studies on *M. natalensis* have mainly focused on the study of hemoglobin patterns: in Southern Africa (South Africa, Zimbabwe, Namibia), GORDON (1978) and GREEN *et al.* (1980) have shown that a «slow» Hb allele was associated with the 32-karyotype. The same type of study was performed on 32-chromosome *Mastomys* from Sierra Leone (ROBBINS *et al.*, 1983) and Nigeria (DOBROKHOTOV, 1982), but without specifying their aFN (see above). The analysis at 20 loci of a sample of *M. natalensis* from Senegal by DUPLANTIER *et al.* (1990b), showed a rather low genetic variability in this species when compared to *M. erythroleucus* and *M. huberti*, and no diagnostic loci between these 3 species. MILISHNIKOV *et al.* (1992) also found low levels of diversity in a sample of what they called *M. huberti*, but which more likely represents *M. natalensis*.

Morphological and biometrical studies on *M. natalensis* have been performed on specimens from Senegal (DUPLANTIER, 1988; DENYS *et al.*, unpubl. data) and South Africa

(DIPPENAAR *et al.*, 1993). Only discriminant analysis on skull and dental measurements enabled the complete or nearly complete characterization of *M. natalensis*. The sperm morphology of *M. natalensis* was very similar to that of *M. coucha* (BREED, 1995; BASKEVICH & LAVRENCHENKO, 1995), whereas the penis and bacculum morphology of *M. natalensis* was distinct from that of *M. erythroleucus* and *Mastomys* sp. from Ethiopia (LAVRENCHENKO & BASKEVICH, 1996).

M. huberti (Wroughton, 1908)

To this species described from Northern Nigeria, PETTER (1977) referred the specimens with 32 chromosomes from Western and Central Africa. Since then, the situation has proven to be more complicated, two species with this 2n (but with different aFN) having been characterized in this region (or at least part of it – DUPLANTIER *et al.*, 1990a). One of them is *M. natalensis*, as stated above, and the specimens with the other aFN probably belong to *M. huberti*. The standard karyotype of this species has been presented by HUBERT *et al.* (1983), the G-banded one by VIEGAS-PEQUIGNOT *et al.* (1983). DUPLANTIER *et al.* (1990a) and BRITTON-DAVIDIAN *et al.* (1995), working on a larger sample, identified its chromosomal variability: 2n=32, aFN=44-46. This chromosomal form, which for a long time was known only from Senegal, has been recently confirmed in Mauritania (GRANJON *et al.*, 1997), Mali and Burkina-Faso (SICARD, pers. comm.). This last finding supports the belonging of these specimens to *M. huberti*, a decision which will be definitely validated when specimens from the type region are studied. Unless there is a spectacular range extension of this form towards East Africa, we see no reason to refer it to *M. hildebrandtii*, the type specimen of which was described from Kenya.

Chromosomally characterized *M. huberti* have been studied by protein electrophoresis (DUPLANTIER *et al.*, 1990b) and biometrical analyses (DUPLANTIER, 1988; DENYS *et al.*, unpubl. data). None of these methods led to the finding of any clear diagnostic character for distinguishing *M. huberti* from its sympatric congeneric species (*M. erythroleucus* and *M. natalensis*).

M. pernanus (Kershaw, 1921)

This species, characterized by its small size, is only known from a few specimens and raptor pellet remains from N.W. Tanzania, S.W. Kenya and Rwanda (MISONNE & VERSCHUREN, 1964). The latter authors proposed to maintain this species within *Mastomys*, but this decision was questioned by ROBBINS & VAN DER STRAETEN (1989) who stated that it may belong to the taxon *Myomys*.

M. verheyeni (Robbins & Van der Straeten, 1989)

This recently described species is still only known from the «Nigeria and Cameroon savanna immediately surrounding the Southern part of Lake Chad» (by ROBBINS & VAN DER STRAETEN, 1989). At present, it has only been studied morphologically and biometrically, and is mainly characterized by its large size.

DISCUSSION

Some authors have made an attempt to elucidate phylogenetic relationships within *Mastomys*, but in no instance have all the species cited above been taken into account. A preliminary analysis by GORDON (1985), based on chromosomal data, distinguished two groups: one with *M. coucha* and *M. shortridgei* (i.e. the species with 2n=36), with *M. erythroleucus* (2n=38) as the sister species, and the other with *M. natalensis* and *M. huberti* (i.e. the species with 2n=32). Later, CHEVRET *et al.* (1994) using DNA/DNA hybridization were not able to resolve the relationships between *M. huberti*, *M. erythroleucus* and *M. natalensis*, *M. coucha* representing a possible sister species of the three others. They proposed a date of divergence of 0.3 Myr for the first three species, whereas *M. coucha* could have diverged 1.0 Myr ago. Finally, BRITTON-DAVIDIAN *et al.* (1995) performed a phylogenetic analysis of chromosomal characters based on parsimony on the same 4 species, using *Myomys daltoni* (Thomas, 1892) and *Praomys tullbergi* (Thomas, 1894) as outgroups. They suggested that *M. natalensis* and *M. huberti* were the most derived taxa, from a chromosomal point of view. According to this analysis, chromosomal evolution in the genus would have proceeded by i) changes in diploid number by fusion-fission events, and ii) modification of aFN mainly through pericentric inversions. The phylogenetic relationships inferred from these 3 studies are represented on Fig.1.

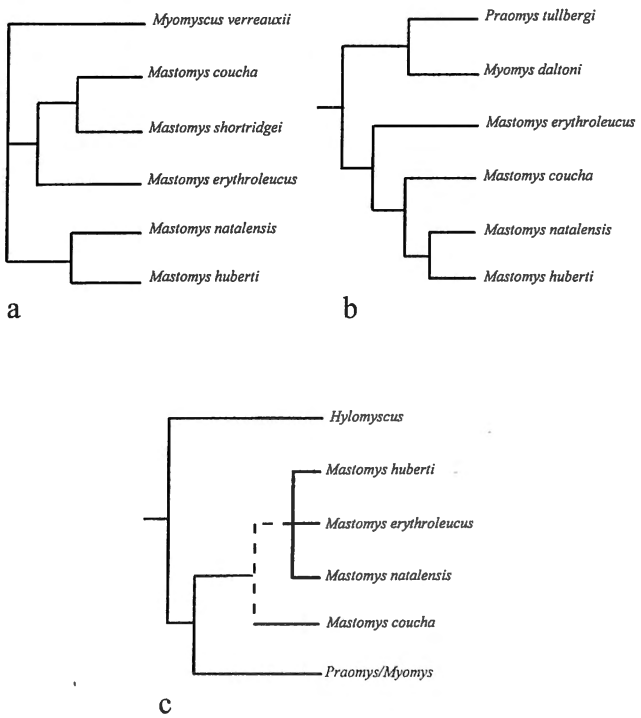


Fig. 1. – Phylogenetic relationships in *Mastomys* and related genera, according to a) GORDON (1985); b) BRITTON-DAVIDIAN *et al.* (1995); c) CHEVRET *et al.* (1994).

These data highlight the fact that, as in many other African rodent genera (MEESTER, 1988), species diversification in *Mastomys* has occurred recently (i.e. within the last million of years or so), and has been accompanied by extensive chromosomal rearrangements. The morphological and genetic divergence between the species is relatively small which explains the difficulties in recognition and characterization of the different species. Karyological studies appear as an especially informative method for species identification, and have yielded clear diagnoses of species such as *M. erythroleucus*, *M. coucha*, *M. nata-*

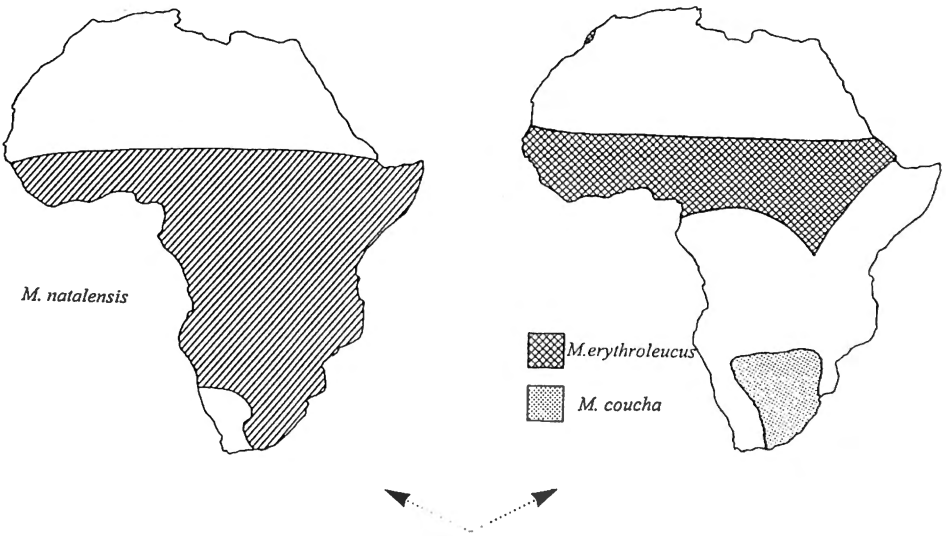
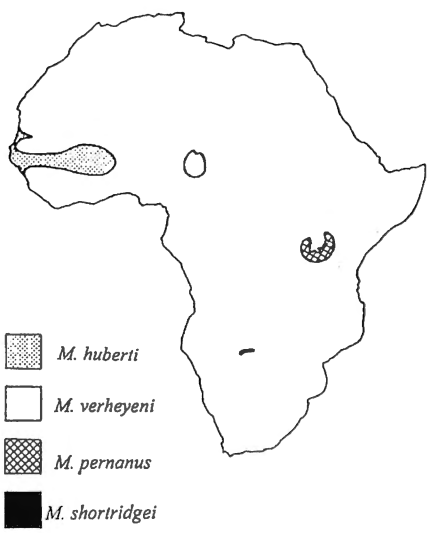


Fig. 2. – Distribution maps
of the different species of *Mastomys*



lensis and *M. huberti*. The precise status of *M. shortridgei* still needs to be ascertained and will require other types of analysis (protein electrophoresis, DNA sequencing...) as its karyotype is closely related to that of *M. coucha*. A combination of various techniques will also be necessary to further define the status of *M. verheyeni* and *M. pernanus*, and of *Myomys* (?) *angolensis*, as well as to identify additional cryptic species in the genus, among which some of the chromosomal variants with 38 or 36 chromosomes (see above) are good candidates. Another problem concerns the confirmation of an East African species which may be *M. hildebrandtii*. QUMSIYEH *et al.* (1990) presented a karyotype for Kenyan specimens with 2n=32, aFN=50-54, that they consider different from *M. natalensis*. Similarly, LAVRENCHENKO *et al.* (1992), BASKEVICH & ORLOV (1993) and LAVRENCHENKO & BASKEVICH (1996) have described specimens of *Mastomys* sp. from Ethiopia, also carrying 32 chromosomes, but that they consider as belonging to a species distinct from *M. natalensis*. However, the evidence is still not convincing, and further studies should try to definitely characterize and name this species, and more precisely to define its species limits when compared to true *M. natalensis* (as was done in West Africa between *M. huberti* and *M. natalensis*, as described above).

Finally, the distribution of all these species has to be precisely determined, the maps given in Fig. 2 representing only preliminary attempts based on the data available to date. Only in a few cases (DUPLANTIER & GRANJON, 1988, for Senegal; SKINNER & SMITHERS, 1990, for Southern Africa) have the distribution areas of the *Mastomys* species been mapped on a larger scale. These biogeographical aspects will represent one important by-product of the development of new methods of species discrimination, and of the application of genetic (and especially chromosomal) techniques on specimens from throughout the genus range.

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AFRICAN ARENAVIRUSES – COEVOLUTION BETWEEN VIRUS AND MURID HOST?

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Abstract. The Arenaviridae is a family of enveloped, negative-stranded RNA viruses which cause severe hemorrhagic fever in humans in areas of Africa and South America. Each arenavirus is generally associated with a single small-mammal host species in which it establishes a chronic infection involving shedding of virus in secretions and excretions. Infection in humans occurs via inhalation of aerosolized virus or ingestion or direct contact with food or fomites contaminated with infectious animal wastes. Genetic analysis shows that known arenaviruses fall into a New-World and an Old-World complex. New-World arenaviruses are associated with species of the rodent subfamily Sigmodontinae and Old-World viruses with the subfamily Murinae. This pattern suggests that an ancestral arenavirus was associated with an ancestral murid rodent before the two subfamilies diverged > 20 million years ago, and that distinct arenaviruses may have coevolved with murid species since that time. If this hypothesis is true, the phylogeny of the arenaviruses should mirror the phylogeny of their rodent hosts. Although the prediction of coincidence of host and virus phylogenies is supported for another group of viruses with murid hosts (the hantaviruses), a comparison of arenavirus and host phylogenies reveals several important inconsistencies. These irregularities may reflect cross-taxon transfer of viruses as well as the relatively incomplete knowledge of the systematics of African and South American murids. Doubtless, many more arenavirus/host associations remain to be discovered within Africa. Continued studies of the relationships among African murids and collaboration between mammalogists and virologists are important to the development of both disciplines.

Key words: Arenavirus, Arenaviridae, hemorrhagic fever, Lassa fever, coevolution, *Mastomys*.

INTRODUCTION

The Arenaviridae is a family of enveloped, negative-stranded RNA viruses. Arenaviruses are responsible for severe hemorrhagic fever in humans on two continents. The South American arenaviruses that are pathogenic for humans include Junín, Machupo, Guanarito, and Sabiá viruses. Junín and Machupo viruses are the etiologic agents of Argentine and Bolivian hemorrhagic fevers, respectively. Sabiá virus has been responsible for a single, naturally acquired, fatal case of hemorrhagic fever in São Paulo State, Brazil. Lymphocytic choriomeningitis virus (LCMV) is widespread in both the New and Old Worlds. LCMV usually produces a syndrome of fever, myalgia, and leucopenia, although, occasionally, severe encephalitis may ensue. The only African arenavirus known to cause human disease is Lassa virus, the etiologic agent of Lassa fever. There is also a large group of arenaviruses which are not associated with human disease, for a total of

TABLE 1

Arenaviruses associated with rodent hosts of the family Muridae

Virus	*Host*	*Known Distribution of Virus*	*Disease*	*Reference*
A. Subfamily Murinae				
Lymphocytic choriomeningitis	*Mus musculus* L., 1758	Europe, Americas, perhaps worldwide	Lymphoctic choriomeningitis	ARMSTRONG & LILLIE 1934
Lassa	*Mastomys* spp.	West Africa	Lassa fever	FRAME *et al.* 1970
Ippy	*Arvicanthis* spp.?	Central African Republic	None recognized	SWANEPOEL *et al.* 1985
Mopeia	*Mastomys natalensis* (Smith, 1834)	Mozambique, Zimbabwe	None recognized	WULFF *et al.* 1977
Mobala	*Praomys jacksoni* (De Winton, 1897)	Central African Republic	None recognized	GONZALEZ *et al.* 1983
B. Subfamily Sigmodontinae				
Junìn	*Calomys musculinus* (Thomas, 1913)	Central Argentina	Argentine hemorrhagic fever (AHF)	PARODI *et al.* 1958
Machupo	*Calomys callosus* (Rengger, 1830)	Beni Department, Bolivia	Bolivian hemorrhagic fever (BHF)	JOHNSON *et al.* 1965
Guanarito	*Zygodontomys brevicauda* (Allen and Chapman, 1893)	Central llanos, Venezuela	Venezuelan hemorrhagic fever (VHF)	SALAS *et al.* 1991 FULHORST *et al.* in press
Sabiá	Unknown	Near Sao Paulo, Brazil	Not named	COIMBRA *et al.* 1994
Amaparì	*Neacomys guianae* (Thomas, 1905) *Oryzomys capito* (Olfers, 1818)	Amapa Territory, Brazil	None recognized	PINHEIRO *et al.* 1966

Flexal	*Oryzomys* spp.?	Pará State, Brazil	None recognized[a]	PINHEIRO *et al.* 1977
Latino	*Calomys callosus*	Beni Department, Bolivia	None recognized	WEBB *et al.* 1973
Oliveros	*Bolomys obscurus* (Waterhouse, 1837)	Central Argentina	None recognized	MILLS *et al.* 1996 BOWEN *et al.* 1996
Paraná	*Oryzomys buccinatus*? (Olfers, 1818)	Missiones Province Paraguay	None recognized	WEBB *et al.* 1970
Pinchindé	*Oryzomys albigularis* (Tomes, 1860)	Columbia	None recognized	TRAPIDO & SANMARTIN 1971
Pirital	*Sigmodon alstoni* (Thomas, 1881)	Central Llanos, Venezuela	None recognized	FULHORST *et al.* in press
Tacaribe	Unknown[b]	Trinidad	None recognized[a]	DOWNS *et al.* 1963
Tamiami	*Sigmodon hispidus* (Say and Ord, 1825)	South Florida	None recognized	CALISHER *et al.* 1970
Whitewater Arroyo	*Neotoma albigula* (Hartley, 1894)	SW USA	None recognized	FULHORST *et al.* 1996 KOSOY *et al.* 1996

[a] One documented laboratory infection

[b] Original report lists bats from the genus *Artibeus* as the reservoir; subsequent attempts to isolate virus from *Artibeus* have been unsuccessful

19 currently recognized arenaviruses (Table 1). Fourteen are New World viruses (the Tacaribe Complex), and five are from the Old World Lassa virus (LASV)-LCMV complex.

A hallmark of the arenaviruses is their association with a single host species of the rodent family Muridae (Table 1), in which they establish a chronic, persistent infection that involves the shedding of infectious virus in urine, feces, and saliva. Possible exceptions to this rule are Sabiá virus, whose reservoir is unknown, and Tacaribe virus, which has been isolated only from bats of the genus *Artibeus*. It is questionable, however, that bats are the true reservoir for Tacaribe virus.

Rodent-to-rodent transmission of arenaviruses can occur either vertically (from parent to offspring) or horizontally (from adult to adult). For example, there is strong evidence from field studies that Junín virus is transmitted horizontally, perhaps among adult male animals during aggressive encounters (MILLS *et al.*, 1992). Conversely, Lassa virus and LCMV appear to be transmitted vertically, perhaps transplacentally (MIMS, 1975).

Human infection results from inhalation of viral particles contained in aerosols of infectious rodent excretions and secretions. Infection may also result from direct contact of broken skin or mucous membranes with contaminated objects or, possibly, via the ingestion of food contaminated by infected rodents (PETERS *et al.*, 1996). Person-to-person transmission of arenaviruses is not common, but has been documented, especially with Lassa fever and Bolivian hemorrhagic fever. Person-to-person transmission likely results from direct contact with bodily fluids of patients, from needle-stick injuries, or from reuse of nonsterile needles and syringes.

THE ARENAVIRIDAE

History

The prototype arenavirus, LCMV, was discovered in 1933 by researchers studying samples from an epidemic of St. Louis encephalitis (ARMSTRONG & LILLIE, 1934). LCMV was not the cause of the epidemic, but was later discovered to be responsible for non-fatal aseptic meningitis in humans as well as a chronic infection in colonies of laboratory mice. This finding led to the discovery of the association of LCMV with its natural host, the cosmopolitan house mouse, *Mus musculus*. Argentine hemorrhagic fever was first recognized in 1953 (ARRIBALZAGA, 1955), and the etiologic agent, Junín virus, was described in 1958 (PARODI *et al.*, 1958). Bolivian hemorrhagic fever was described after an outbreak in northeastern Bolivia in 1959, and the etiologic agent, Machupo virus, was isolated a few years later (JOHNSON *et al.*, 1965). Guanarito virus was isolated after an outbreak of hemorrhagic fever in Venezuela (SALAS *et al.*, 1991). Sabiá virus is known from a single naturally acquired human case, near Sao Paulo, Brazil in 1990 (COIMBRA *et al.*, 1994). Since then, there have been two additional cases due to laboratory infection with Sabiá virus (VASCONCELOS *et al.*, 1993; BARRY *et al.*, 1995).

African arenaviruses

In 1969, Lassa virus was isolated from human patients during an investigation of severe hemorrhagic fever in missionary nurses in Nigeria (FRAME *et al.*, 1970). The host

of Lassa virus was unknown until 1972, when it was isolated from *Mastomys* trapped in Sierra Leone (MONATH *et al.*, 1974). Ippy virus was isolated from *Arvicanthis* in the Central African Republic very soon after the discovery of Lassa virus (DIGOUTTE, 1970), but was not shown to be related to Lassa virus until 15 years later (SWANEPOEL *et al.*, 1985). Mopeia virus (formerly known as Mozambique virus), isolated from *Mastomys natalensis* captured in central Mozambique in 1972, was also shown to be related to Lassa virus (WULFF *et al.*, 1977). The most recently discovered member of the complex is Mobala virus, which was isolated from *Praomys* species captured in the Central African Republic (GONZALEZ *et al.*, 1983).

Lassa Fever

Although accurate figures on the incidence of the disease do not exist, Lassa fever is a serious public health problem in West Africa. Thousands of clinical cases occur each year in Sierra Leone, Guinea, Liberia, and Nigeria, with a mortality of about 15% (PETERS *et al.*, 1996). After an incubation period which averages 10 days, Lassa fever has a gradual, insidious onset with fever and malaise, followed by muscle aches and prostration. Gastrointestinal symptoms, including nausea, vomiting, diarrhea, or even constipation, are common, as are sore throat and pharyngitis. Hemorrhagic symptoms are much less common than in the South American hemorrhagic fevers (PETERS *et al.*, 1996). Associated complications include spontaneous abortions and unilateral or bilateral deafness, which may be temporary or permanent (CUMMINS *et al.*, 1990).

Aggressive supportive treatment is important in the management of patients with Lassa fever. Intravenous administration of an antiviral drug, Ribavirin, has been shown to ameliorate symptoms, especially in severe cases (MCCORMICK *et al.*, 1986).

Philogeny of arenaviruses

A phylogenetic analysis of the *Arenaviridae* was recently performed using nucleotide sequences of the nucleocapsid gene (Fig. 1; BOWEN *et al.*, in press). The known arenaviruses fall into two major groups, the New-World Tacaribe complex and the Old-World LASV-LCMV complex. The Tacaribe complex can be further divided into three subgroups, with all of the important human pathogens falling into the same subgroup (Group B, Fig. 1).

The LASV-LCMV complex also can be divided into three subgroups. LCMV and Ippy virus occupy basal lineages; Lassa, Mobala, and Mopeia viruses

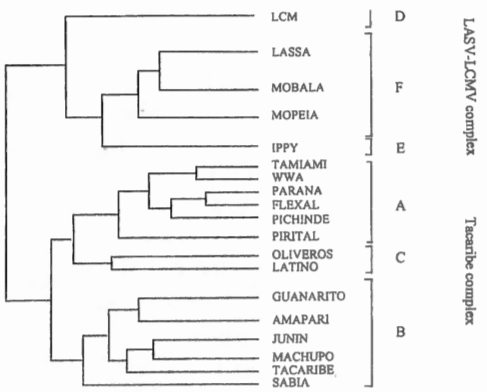

Fig. 1. – Phylogenetic relationships among the Arenaviridae (after BOWEN *et al.*, in press). LCM=lymphocytic choriomeningitis; WWA=Whitewater Arroyo.

appear monophyletic and considerable sequence divergence exists within both Lassa and Mopeia viruses. This sequence divergence may indicate that different viral subtypes are associated with different rodent taxa within the poorly understood *Mastomys natalensis* complex (BOWEN *et al.*, in press).

A recent taxonomic reference (MUSSER and CARLETON, 1993) names 8 species of *Mastomys*, but notes that the genus requires careful taxonomic revision. Four morphologically similar species (formerly all considered as *M. natalensis*) have been now separated on the basis of chromosomal traits (DUPLANTIER *et al.*, 1990). These include *M. coucha* (Smith, 1834), characterized by 2N=36 (FN=56); *M. erythroleucus* (Temminck, 1853), 2N=38 (FN=52); *M. hildebrandtii* (Peters, 1878), 2N=32 (FN=44); and *M. natalensis* (Smith, 1834), 2N=32 (FN=54). Mopeia virus is described from *M. natalensis* in Mozambique. Although *M. natalensis* was originally described as the reservoir of Lassa virus, it may be that the species involved in the maintenance of Lassa virus in West Africa are *M. erythroleucus* and *M. hildebrandtii*.

EVIDENCE FOR COEVOLUTION

With the exception of Tacaribe virus, all the arenaviruses with known reservoirs are associated with rodents of the family Muridae (Table 1). The New-World viruses are hosted by rodents of the subfamily Sigmodontinae (New-World rats and mice) and the Old-World viruses by rodents of the subfamily Murinae (Old-World rats and mice). This pattern suggests that an ancestral arenavirus was associated with an ancestral murid and that the distinct arenaviruses have been coevolving with the various species of murids since before the two groups diverged more than 20 million years ago. If this is true, one would expect to see a high degree of similarity between the phylogeny of the rodents and that of the viruses (i.e., viruses associated with closely related rodents should, themselves, be closely related and vice versa).

Clear evidence for coevolutionary relationships is seen when viral phylogeny is compared with host taxonomy for another group of viruses which have murid hosts, the hantaviruses (GLASS *et al.*, in press; HJELLE et al., 1995; NICHOL et al., 1996). One clade of viruses is associated with the arvicolines in both the Old and New Worlds, another with the sigmodontines in the New World, and yet another with the Old-World murines. Even within subfamilies of rodents, closely related genera and species are associated with genetically similar viruses. Finally, a distinct virus (Thottopalayam) is associated with the insectivore, *Suncus murinus*. This close correspondence of phylogenetic patterns indicates that the phylogeny of the viruses might provide clues into the phylogenies of their hosts.

The phylogenetic relationships among the murid rodents are still the subject of much debate. The topology we have depicted (Fig. 2) follows that proposed by STEPPAN (1995) for sigmodontine rodents and CHEVRET (1994) for murine rodents. For simplicity, only rodent tribes and genera which host arenaviruses (and a few important type genera) are depicted. The sigmodontines are generally divided into the North American and South American lineages, and the South American sigmodontines are generally divided into seven or eight "tribes" of purportedly related species (Fig. 2). Among the African genera, *Arvicanthis* is depicted in a separate clade from *Mus*, *Praomys*, and *Mastomys*. *Mastomys*

and *Praomys* are poorly differentiated morphologically and are usually treated as closely related genera or subgenera within *Praomys*.

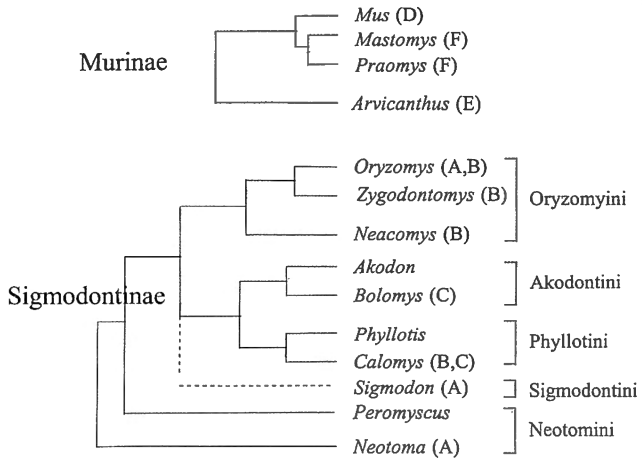

Fig. 2. – Phylogenies of the rodent hosts of arenaviruses (Sigmodontinae after STEPPAN, 1995), Murinae after CHEVRET, 1994). Letters in parentheses refer to viral clades depicted in Fig. 1. The dotted line to *Sigmodon* reflects the unclear position (Sigmodontinae *incertae sedis*, STEPPAN, 1995) of that genus in the three.

An attempt to make similar phylogenetic comparisons among arenaviruses and their hosts is much less successful than with the hantaviruses (Fig. 2). The distant relationship between the viruses of the Tacaribe Complex and the LASV-LCMV complex reflects the distant relationship between the murines and the sigmodontines but, on a finer scale, any coevolutionary relationships are obscure. In the New World, there are viruses of group A among three diverse tribes of rodents, and two genera serve as reservoirs for viruses from two different clades. In Africa, *Mastomys* apparently hosts two different arenaviruses, and Ippy virus is associated with a rodent species only distantly related to the *Mastomys/Praomys* complex. It is quite possible that some of the lack of concordance in phylogenies results from cross-taxon transfer of viruses (spillover of viruses into unrelated hosts may have resulted in the chance establishment of relatively recently derived host-virus relationships). Nevertheless, it is likely that much of the apparent lack of concordance is due to incomplete knowledge of rodent systematics among the South American and African murids, and incomplete knowledge of African arenaviruses.

Given the diversity of arenaviruses in the Americas, it is likely that there are many more arenaviruses in Africa which remain to be discovered. The presence of arenaviruses in both sigmodontine and murine rodents, and the possible specific coevolution between viruses and hosts implies the potential existence of an arenavirus for each murid host species. Past failure to detect human diseases which are related to these viruses is not evidence that they do not exist or that any viruses which remain to be discovered are non-pathogenic for humans. The fact that hantavirus pulmonary syndrome was not discovered

in the United States until 1993 (NICHOL *et al.,* 1993; CHILDS *et al.*, 1994) demonstrates that deadly pathogens associated with common and widespread host species can go undetected for many years, even in a country with a relatively advanced disease surveillance system. No systematic search for arenaviruses in murid hosts has been undertaken, yet an opportunistic screening of samples collected for another purpose recently yielded a new arenavirus from the southwestern United States (Whitewater Arroyo virus; KOSOY *et al.*, 1996; FULHORST *et al.*, 1996).

CONCLUSIONS

The complex of African arenaviruses is certain to be much larger than is currently recognized. Unless they are actively sought, these viruses may go unnoticed for many years, even though they may include important human pathogens. Finding and describing these viruses will require collaborative studies between virologists and rodent ecologists. Understanding the phylogenetic and ecologic relationships among the hosts and viruses will require continued studies of the phylogeny and ecology of African murids. Continued and increasing collaboration between mammalogists and virologists will be essential to progress in both fields. The disciplines have much to offer each other.

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SPATIAL DYNAMICS OF *MASTOMYS NATALENSIS* IN A FIELD-FALLOW MOSAIC IN TANZANIA.

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Abstract. The population dynamics of *Mastomys natalensis* rats is reported for a 3-year study of monthly CMR-trapping in a small scale maize field-fallow land mosaic in Tanzania. The seasonal evolution of rodent presence was the same in both habitat types and it was not affected by agricultural activities in the fields. About one week after planting, there was a short increase of rodent captures in the maize fields, but this disappeared again after a few days. Recolonisation of fields was achieved very fast after a rodent control operation. Radiotelemetry indicated that many individuals were active in the maize field as well as in the fallow land. We concluded that, in a small scale set-up, the field and fallow land rodent populations are not separated and the latter should not be considered as necessary refuges. This renders several rodent control approaches unsuitable.

Key words: rodents, population dynamics, refuge habitat, pest control, Africa.

INTRODUCTION

Agricultural fields are situated in a matrix of surrounding habitat which, in an African smallholder setting, is most often fallow land. Since habitat quality of the fields is dramatically changing during the year, it can be expected that the population dynamics of organisms living there will contain an important spatial component. Rodents that are causing damage in the fields may be expected to leave the fields periodically and return only during the attractive crop stages. Such movements are often assumed to be an important process in rodent damage in field crops (*e.g.* FIEDLER & FALL, 1994) but rarely has this assumption been supported by data on movements of individuals rodents. In African rats of the genus *Mastomys*, seasonal invasion of the fields has been long assumed (*e.g.* HARRIS, 1937; ROBERTSON, 1938; FIEDLER, 1988). Yet, the only well documented instance of *Mastomys* sp. population movements originates from temporarily flooded habitats (SHEPPE, 1972).

In the present paper, we investigate population dynamics of *Mastomys natalensis* (Smith, 1834) in a mosaic of fallow land and agricultural fields in Tanzania. We test the hypothesis that the rodent populations in the fields are temporary and die out or dissipate in the fallow land periodically and we verify whether agricultural activities affect the population dynamics of rodents in the fields. Also, we examine experimentally how quickly rodents from surrounding populations in fallow land recolonise maize fields following rodent control operations.

MATERIAL AND METHODS

The study site was located on the campus of the Sokoine University of Agriculture in Morogoro, Tanzania (06°51'S 37°38'E). The population ecology of *M. natalensis* at this site has been described extensively (see LEIRS, 1994). In April 1994, we selected a 100 m x 300 m area of fallow land containing two plots of maize; a central 70 m x 70 m field which was purposedly ploughed and a corner (approximately 30 m x 70 m) in which local farmers penetrated as part of a larger maize field; we later took over the maize field in the corner ourselves (Fig. 1). All other sides of the area were separated from other fields by a zone of at least 30 m of fallow land. The size of the maize fields in our study area is common for smallholder farms in Tanzania.The maize fields were planted in a standard way (planting lines 1 m apart, plant holes 50-60 cm apart, three seeds per planting hole) after heavy rainfall in April and October 1994, April and November 1995 and March 1996. Seeds germinated in all planting seasons but only the crops planted in March-April received enough rainfall to produce a harvest. In late October 1996, the whole area was burned by a bushfire.

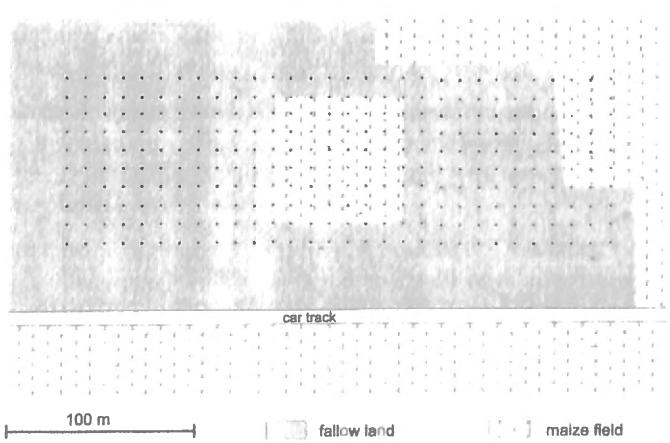

Fig. 1. – Schematic overview of the study area with maize fields and fallow land. Trap stations (dots) are situated in a 10 m x 10 m grid pattern.

A grid was laid out of 30x10 trapping stations, each 10 m apart. Out of 300 trapping stations, 54 were situated in the maize fields, at least 5 m from the border of the fields. We

trapped every month for three consecutive nights with one Sherman trap per trapping station, baited with a mixture of peanut butter and crushed maize. Additional trapping sessions were organised in the week before and after each planting and harvesting session. The present study extended from April 1994 and March 1997. Captured animals were marked individually by toe-clipping; trapping station number, weight and sexual condition were recorded; afterwards each animal was released at its trapping station. We did this for all species of small mammals captured in the study area, but here we report only on M.natalensis. We used trapping success index, i.e. number of individuals trapped per trap night and adjusted for trap saturation as suggested by CAUGHLEY (1977), to assess the presence of animals in the different habitats.

Information on movement patterns was obtained from telemetry, using 2g transmitters from Televilt (Sweden) and Biotrack (UK). We radiotagged a total of 88 *M.natalensis* individuals, trapped after planting time (in March-April and November 1995 and March and May 1996). In November, nearly all tagged animals were trapped in the maize field (39/41) but in March-May (both years pooled), low densities necessitated also captures in the fallow land near the borders of the field and only 25/47 animals were trapped in the field itself. Activity status (moving/not moving) and positions (to the nearest metre) of the tagged animals were recorded several times per night, using a Televilt RX8910HE receiver with a built-in foldable 2 element YAGI-antenna; observations were made every night during periods of three hours; the timing of this three hour period was changed nightly. For each individual, all telemetric localisations were obtained within a three-week period. Spatial concentrations of activity were deduced for each individual from isoplethe shapes in a kernel analysis, carried out in Ranges V (KENWARD, 1996). Since we were interested in movements between habitats, we were not concerned about sample-size dependence of home range and used all individuals for which we had at least two localisations at different positions.

A recolonization experiment was carried out in March 1995. The two maize fields in our permanent CMR-grid were used as controls; for this purpose, we enlarged the corner field to a 70 m x 70 m field since the large field was not yet in use at that moment; two additional 0.5 ha-plots were selected at less than 100 m from the area. All plots were surrounded by at least 30 m fallow land bushes and tall grass. Animals were trapped during one night, marked and released. Maize was planted in all fields on 6 March; in the two experimental fields, a poisoning operation was carried out on the planting day by distributing 1.5 % zinc phosphide in a mixture of maize scrap and cooking oil in small heaps (approx. 20g) every 10 m in the field and extending 10 m in the surrounding fallow land. Carcasses picked up the following morning and autopsied confirmed the efficacy of the poisoning operation. On the day after planting, one night of live-trapping (releasing the captured individuals) was organised in all four fields with 100 Sherman traps per field and this life trapping was repeated four more times within the following two weeks.

RESULTS

In total we realised 35840 trap-nights, resulting in 11470 captures of 4355 individuals; 86% of these were *Mastomys natalensis*, 8.5 % *Tatera robusta* (Cretzschmar, 1830) and

5.5% *Lemniscomys rosalia* (Thomas, 1904); we trapped a single *Mus minutoides* Smith (1834) and 11 shrews *Crocidura* sp.

Trapping success varied greatly throughout the year (Fig. 2). In most trapping sessions, trap success was higher for rats in the fallow land than for those in the maize fields (Sign test, $Z=3.349$, $p<0.001$) but the differences were small and not consistent. There was no increased trapping success in the maize fields in comparison with the fallow land, during or just after the planting week. Trapping success index did differ significantly between seasons with and without crop on the fields ($F=14.34$, $p<0.001$), but not between both habitat types ($F=0.32$, $p=0.573$) and there was no interaction season-habitat ($F=0.02$, $p=0.882$, 2-way ANOVA). There was no obvious effect of the bushfire in October 1996, but we have no control treatment in this «natural» experiment.

We trapped 1071 individuals at least once in the maize fields but 480 of them were trapped also in the fallow land. Documented moves from the fallow land to the maize fields (animals that were trapped for the first time in the maize fields after a previous capture history in the fallow land, *i.e.* the movement direction which is relevant for the study of invasion of the fields) followed a similar seasonal pattern as overall abundance (Fig. 2).

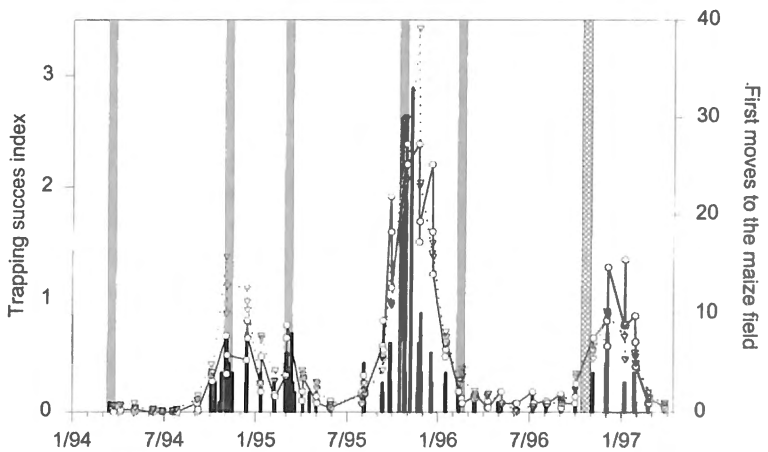

Fig. 2. – Trapping success for each trapping day in the maize field (solid line, solid circles) and fallow land (dotted line, open triangles) between March 1994 and March 1997. The thick grey vertical lines indicate planting periods. The column in October 1996 indicates a savanna fire which destroyed the grass layer in the study area. The narrow black columns indicate the number of individuals that moved from fallow land to maize field.

The number of transient animals (animals that were trapped only once in the study) increased during the October planting seasons, but this happened both in fallow land and in maize fields (Fig. 3). During the March planting season, this increase was not observed. The proportion of transients was higher ($\chi^2=24.5$, $p<0.001$) among animals that were only trapped in the maize field (369/591=62 %) than among animals that were only trapped in the fallow land (1360/2656=51 %). Reproductive activity was highly seasonal but similar in both habitat types (Fig. 4).

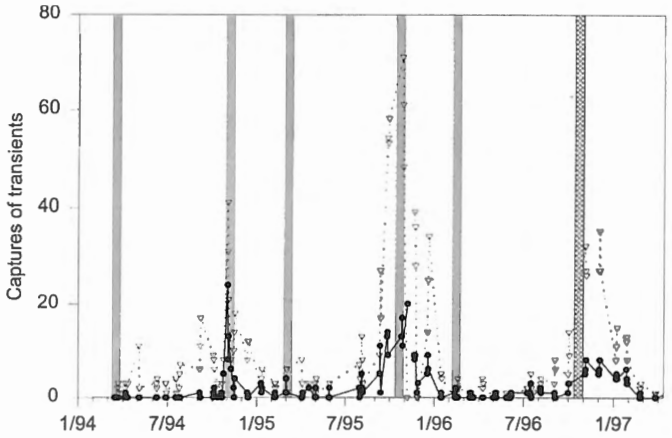

Fig. 3. – Number of transient animals (animals trapped only once) for each trapping day in the maize field (solid line, solid circles) and fallow land (dotted line, open triangles). The thick grey vertical lines indicate planting periods.

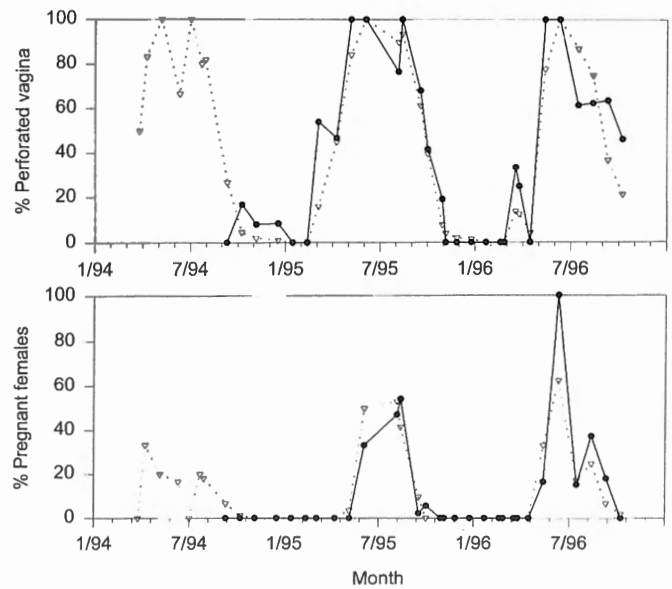

Fig. 4. – Percentage of females with perforated vagina (upper graph) or visible pregnancies (lower graph) per month in the maize field (solid line, solid circles) and fallow land (dotted line, open triangles).

Number and location of activity concentrations could be calculated for 63 of the radio-tagged animals. The 25 other animals died or disappeared before enough telemetric localisations could be collected. Many animals showed a bi- or even polynuclear activity range. In such cases, most animals displayed activity concentrations in the maize fields as well as in the surrounding fallow land (Table 1). This pattern was more obvious when analyses

were restricted to localisations during which the individual was moving (Table 1). There was no relation between the number of activity centers and the total number of localisations for an individual (p=0.25) or the total length of the period during which an individual was followed telemetrically (Spearman Rank correlation, p=0.40). There are relatively more activity concentrations in the maize fields in November, but this corresponds to the bias in capture place of the tagged animals in that month.

TABLE 1

Number and habitat distribution of spatial activity concentrations for 88 radiotagged Mastomys natalensis, *trapped in the maize fields or on the maize field borders in March-May or November (different years pooled). All positions= activity ranges calculated with all telemetric localistations; Moving positions only= activity ranges calculated only with localisations when animals were clearly moving around.*

		Number of individuals			
		All positions		*Moving positions only*	
# activity centers	*Habitat*	*Mar.-May*	*Nov.*	*Mar.-May*	*Nov.*
1	Fallow	7	4	5	3
	Maize	4	12	2	8
≥2	Fallow only	3	2	4	1
	Maize only	2	7	3	8
	Fallow+Maize	6	18	6	19
Insufficient data	-	14	11	15	14

Prior to the recolonization experiment, rat densities were low in all fields (Fig. 5). One day after planting, densities dropped in all fields but in the untreated areas, they started

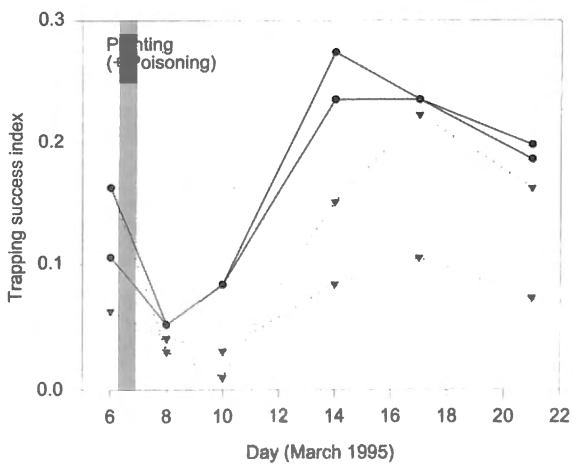

Fig. 5. – Evolution of trapping success in 2 fields where planting was accompanied by a poisoning action (dotted line, triangles) and 2 control fields (solid line, circles). All fields were planted on 6 March (grey vertical column).

increasing again immediately. In the poisoned fields, numbers remained low for at least three days. After that period, densities became high again, both in the treated and untreated fields, although one of the treated fields always remained at a relatively low level. In the untreated field, peak densities were reached at day 14 and they were higher than in the treated fields (one-sided t-test after arcsine-transformation, $p=0.037$).

DISCUSSION

On a seasonal time scale, we found no differences in the amount of rodent activity in fallow land and maize fields. There were animals in the maize fields throughout the year and their relative abundance varied seasonally just like that in the surrounding fallow land. Neither did we find indications of seasonal mass displacements or evidence that the field population disappears or becomes extinct in some seasons and reestablishes itself later on. There is a large turnover in the field population, but the same is happening in the fallow land population. The higher proportion of transients in the maize field could indicate temporary invasions of the fields, even more so because some of the apparent transients in the fallow land could actually be visitors attracted to the fields but intercepted by the traps in the surrounding fallow land. Yet, the seasonal pattern of the presence of transients cannot be explained by the crop seasons. The seasonal effects of rainfall as reported earlier for this population (TELFORD, 1989; LEIRS *et al.*, 1996a) thus seem to be stronger than the effects of agricultural activities or events like savanna fires. The observation that damage by *M. natalensis* in maize fields is highly seasonal and concentrated at planting and harvesting time (TAYLOR, 1968 and many unpublished reports), may therefore be the result of changing feeding behaviour dependent on crop stage, rather than differences in rodent abundance and spatial dynamics.

On a much shorter time scale, within the first week after planting and therefore not obvious in the three-year data series, we did observe effects of agricultural activity in our recolonization experiment. Interestingly, rodent densities in the fields sharply decreased immediately after ploughing and planting, also in the fields where no rodent control was applied, suggesting that the disturbance of the field has an impact, albeit a brief one, on rodent abundance. Numbers, however, then rapidly increased for a few days, reaching a maximum when the germinated seedlings were above the ground, after which they decreased again. We have no similar data on rodent densities in the surrounding fallow land during the same short period, but the fact that we observed the peak and consecutive decline also in fields where the local population was removed at the moment of planting, indicates that indeed a short invasion happened. We have no indications of the long range movements of colonizing animals, but rather long dispersal and excursion distances are not uncommon in *M. natalensis* (LEIRS *et al.*, 1996b; CHRISTENSEN, 1996). Clearly, poisoning would be needed in a band wider than the 10 m we applied around the fields in order to prevent quick recolonisation. This has important consequences for control since widening this band increases the total surface to be treated quadratically, rendering poison treatments economically less profitable. MYLLYMÄKI (1987) already indicated that the dispersal capacities of *M.natalensis* prevented a long-term effect of local control actions, but he suggested that treatments synchronised with planting could protect the seedlings over

a short period. In our experiment, recolonization occurred so quickly, even at low densities, that even a short-term effect seems doubtful.

The short invasion of a field should not necessarily be a move of animals between separate populations. There is a higher proportion of transients in the maize field but that may be biased by the fact that the maize fields have relatively more area close to the border of the trapping area. Most non-transients in the maize fields were trapped at earlier or later occasions in the fallow land and our telemetric data show that individuals regularly moved between both habitats. That most animals spend considerable time in both habitats suggests that rats benefit from some complementarity between maize fields and fallow land. This does not mean that *M.natalensis* are homogeneously distributed at a small scale. It has been shown several times that *Mastomys* sp. individuals prefer sites with specific microhabitat characteristics from studies elsewhere (DIETERLEN, 1967; NEAL, 1970; MARTIN & DICKINSON, 1985; DUPLANTIER & GRANJON, 1988) and from our own study area (TELFORD, 1989; LEIRS *et al.*, 1996b). Observations that fields are first damaged at their borders (*e.g.* EVERARD,1966; TAYLOR, 1968) are not necessarily indications of invasions by rodents, but may be the result of a microhabitat preference of the rodents (near to more lush cover) in their usual home range. Rodent damage is often patchy in the maize fields (unpublished observations) and we observed also that radiotagged animals tend to be localised more in the surroundings of small weedy patches or left-over bushes in the field. Our telemetric data show that many individuals have disjunct activity centres, contrasting with earlier assumptions that *M.natalensis* has a simple circular home range (*e.g.* CHRISTENSEN, 1996).

The invasion of fields by rodents from surrounding populations is a general assumption in rodent pest ecology and several rodent strategies proposed for managing rodent damage have attempted to minimize influx of animals by fencing (*e.g.* SINGLETON, 1997), diversionary feeding (PELZ, 1989), perimeter poisoning (KAY *et al.*, 1994), buffer or capture crops and periphery environmental control (FITZWATER, 1988). Often, however, the evidence for large population movements into fields comes from studies which were carried out in very large scale agricultural landscapes, like the work on house mice in Australia (*e.g.* NEWSOME, 1969; TWIGG & KAY, 1995; KREBS *et al.*, 1995; CHAMBERS *et al.*, 1996) or the invasions of *Arvicanthis niloticus* (Desmarest, 1822) in the sahelian savanna during outbreak years (POULET & POUPON, 1978). When the different habitat patches are smaller, the evidence for seasonal movements is much weaker, a rare exception being the study by CUMMINGS & VESSEY (1994) who showed that individual *Peromyscus leucopus* (Rafinesque, 1818) change habitats seasonally in a small-scale patchy agricultural landscape in Ohio. Our present data indicate that, in fine-grained habitat typical for many African smallholder farms, the distinction between refuge and field populations disappears and consequently it can be assumed that management techniques based on the existence of both spatially and temporarily distinct populations will be of limited use there.

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POPULATION ECOLOGY OF RODENTS OF MAIZE FIELDS AND GRASSLAND IN CENTRAL ETHIOPIA

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Abstract. We report on the presence of rodents in grassland and maize fields in central Ethiopia, during the course of a 21-month study by means of removal and capture-recapture trapping. In both habitats, the small mammal fauna consisted of the same species but in different relative proportions: *Arvicanthis dembeensis*, *Mastomys erythroleucus, Tatera robusta, Rattus rattus, Mus mahomet* and *Crocidura olivieri. A. dembeensis* and *M. erythroleucus* were the dominant species. Densities were generally low throughout the study period, but at the end of the breeding season in the second year of the study, the numbers of *A.dembeensis* reached high values in the grassland. Breeding was seasonal and related to rainfall periods: extended rainy seasons resulting in longer periods with breeding females and higher litter sizes and, consequently, population size increases. These observations suggest that rodent population dynamics in the study area are linked to rainfall patterns and this information can be used to develop forecasting models.

Key words: *Mastomys, Arvicanthis, Tatera*, rodent ecology.

INTRODUCTION

Rodent outbreaks over large areas have been reported from many localities in Africa but also in non-outbreak years damage to agriculture may be considerable (FIEDLER, 1988). In Ethiopia, it has been estimated that rodents consume or destroy up to 20% of the cereal crops in some years (GOODYEAR, 1976). The country possesses 70 rodent species of which 15 are endemic (HILLMAN, 1993; YALDEN & LARGEN, 1992 and YALDEN *et al.*, 1996). Despite this diversity, only few studies on the taxonomy, distribution and population ecology of rodents have been conducted in Ethiopia (INGERSOL, 1968; YALDEN *et al.*, 1976, MÜLLER, 1977; RUPP, 1980; YALDEN, 1988a, b; AFEWORK BEKELE *et al.*, 1993 and AFEWORK BEKELE, 1996) and most of these did not focus on pest species. Preliminary surveys showed that there are eleven species of rodents in Ethiopia that can be classified as pests, among which *Arvicanthis dembeensis* (Thomas, 1901) and *Mastomys erythroleucus* (Temminck, 1853) are the major ones in the field (unpublished information).

A sound understanding of population dynamics may allow to predict changes in rodent numbers, which is of prime importance for the development of management strategies. Recently, an outbreak prediction method using rainfall information was presented for Tanzanian populations of *Mastomys natalensis* (Smith, 1834) (LEIRS *et al.*, 1996); the rela-

tion between rainfall and life histories, mainly reproduction, was central in that study. In order to investigate to what extent the same underlying biological knowledge could be applied to pest rodent populations in other parts of East Africa, we set up a basic ecological study in Ethiopia. Indeed, although the reproductive pattern and ecology of *Mastomys* and *Arvicanthis* species in different regions of Africa have been studied by different investigators (BRAMBELL & DAVIS, 1941; OLIFF, 1953; JOHNSTON & OLIFF, 1954; MEESTER, 1960; COETZEE, 1965; BAKER & MEESTER, 1977; NEAL, 1981; CHIDUMAYO, 1984; LEIRS *et al.*, 1990 and LEIRS, 1995), very little has been done on the Ethiopian species. Here we report the first results of our study, focusing on the seasonality of reproduction.

MATERIAL AND METHODS

The study area (Koka Dairy Farm Enterprise) is located along the main Rift Valley, 95 km South of Addis Ababa, at 08°25'N 39°02'E, 1700 m asl. The vegetation consists of savanna woodland. It was formerly protected as a ranch and at present serves as a centre for hay preparation and maize farm for feeding dairy cattle. The surrounding area is intensively farmed and degraded. Different sites that represent farmland (maize fields) and grassland (fallow land) were selected after assessing the presence of pest rodents.

Data were collected by using both Capture-Mark-Recapture (CMR) and removal trapping techniques, between April 1995 and December 1996. For the CMR study, a one hectare grid in the maize farm and another one in the grassland were laid out. Each grid consisted of 100 trapping stations, 10 m from each other in a square quadrat. Coded wooden pegs or bricks were used at each trapping station. In each grid, we trapped every month with 100 Sherman live traps for three consecutive days, using peanut butter as bait. The traps were checked daily during early morning and late afternoon. However, in summer, the traps were closed from 10.00 a.m to 3.00 p.m. The captured animals were marked by toe-clipping. The date of capture, trapping station, toe-clipping code, sexual condition and body weight (using a Pesola spring balance, accurate to the nearest g) were recorded for each captured animal prior to release. Population sizes were enumerated as Minimum Number Alive (KREBS, 1966) since recapture/release occurrences in several months were too low to allow statistical estimates; for the same reason, we do not discuss survival patterns in this paper.

Monthly removal trapping was initially carried out with Museum Special mouse traps and Victor rat traps. The Museum Special mouse traps were later on avoided because of their inefficiency in capturing larger-sized rodents. The location of trapping sites was changed every month to cover different vegetation zones. However, since March 1996, removal trapping was restricted to maize field and grassland habitats similar to those of the CMR grids; in the present paper we use only the animals from these two habitat types. The captured animals were weighed, standard external measurements were taken and reproductive condition was recorded. The animals were then preserved in formalin and are being kept in the collections of the Zoological Natural History Museum, Addis Ababa University, Ethiopia. Climatological data on rainfall, minimum and maximum temperature were collected on a daily basis since June 1995 from our own small weather station at the

Koka Dairy Farm; the preceding months of the field study (April-May 1995) had little rain (unquantified observations).

RESULTS

The small mammal fauna in the removal trapping consisted of *Arvicanthis dembeensis*, *Mastomys erythroleucus*, *Tatera robusta* (Cretzschmar, 1830), *Crocidura olivieri* (Lesson, 1827), *Mus mahomet* Rhoads (1896) and *Rattus rattus* (L., 1758). In total, the removal trapping yielded 2995 specimens in grassland and maize fields (Table 1). *A. dembeensis* and *M. erythroleucus* were the dominant species, accounting for 87% of the total capture, with *A. dembeensis* being the most common rodent in grassland while *M. erythroleucus* took this position in the maize fields. The species distribution was significantly different between both habitat types ($\chi^2=231.72$, d.f.$=5$, $p<0.001$). In the period between April and December 1996, when trapping effort was the same in both habitat types, less species were trapped but the common species showed an equally different habitat preference ($\chi^2=293.68$, d.f.$=3$, $p<0.001$) with many more *A. dembeensis* trapped in the grassland and *M.erythroleucus* in the maize fields; in total, considerably more specimens were collected in the grassland (Table 1).

TABLE 1

Number of individuals of different species collected during the removal trapping study and CMR-study in grassland and maize field habitats in Koka. The figures in italics indicate the number of specimens obtained between April and December 1996 when the monthly removal trapping effort was the same in both habitats

	Removal study						*CMR-study*		
	Grass		*Maize*		*Total*		*Grass*	*Maize*	*Total*
Arvicanthis dembeensis	1484	*403*	114	*71*	1598	*474*	149	84	233
Mastomys erythroleucus	730	*76*	293	*228*	1023	*304*	68	87	155
Tatera robusta	202	*109*	67	*57*	269	*166*	57	58	115
Mus mahomet	3	-	-	-	3	-	2	3	5
Rattus rattus	5	-	-	-	5	-	0	1	1
Crocidura olivieri	86	*39*	11	*10*	97	*49*	25	23	48
Total	2510	*627*	485	*366*	2995	*993*	301	256	557

In the CMR-study, we realised 1470 captures of 557 individuals. The same species occurred as in the removal trapping and also here the species distribution differed between grids ($\chi^2=18.24$, d.f.$=5$, $p=0.003$). Again *A.dembeensis* was the dominant species in the grassland while *M.erythroleucus* was the most common one on the maize fields (Table 1). The most common rodent species (*A. dembeensis* and *M. erythroleucus*) showed similar fluctuations in population size in the grassland grid (Fig. 1). Although population sizes were low and rather constant during most of the time, both species showed an increase in num-

bers at the end of the rainy season. In 1995, the populations quickly returned to low numbers, but in 1996 they continued to increase well into the dry season and then decreased again towards the end of the year. Although *A. dembeensis* was not trapped during the initial months of the work in the grassland, this species reached high numbers there in late 1996. The population of *T. robusta* in the grassland showed an increasing trend but no such obvious fluctuations. In the maize grid, the situation was different with more irregular patterns (Fig. 1). Here *A.dembeensis* reached its highest densities, but no sharp peaks, in the dry season of 1995-1996; at that moment the species was rare in the grassland. The reverse happened in late 1996. The numbers of *M. erythroleucus* did not fluctuate considerably but showed throughs in the dry season and higher densities during the rainy seasons.

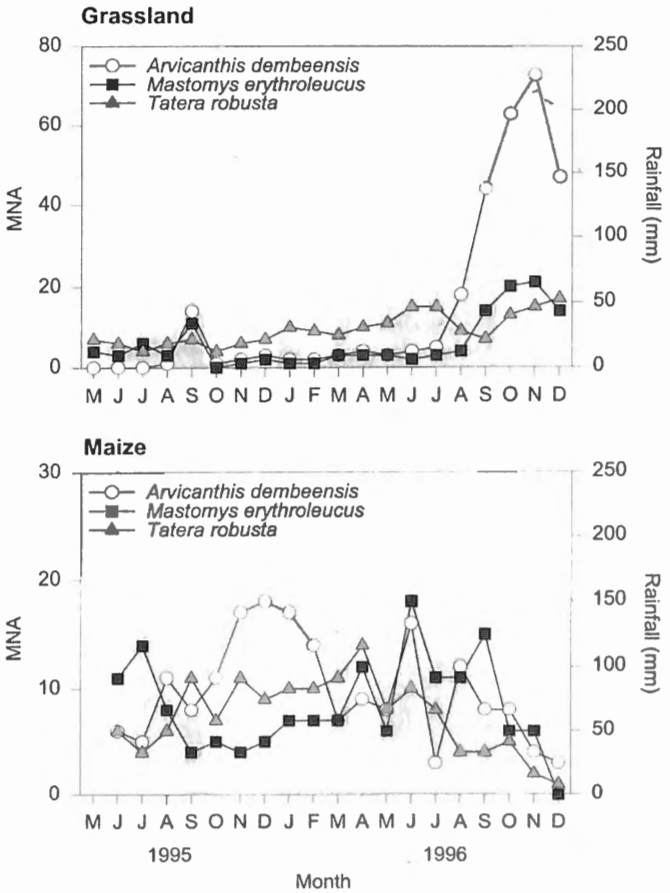

Fig. 1. – Monthly variation of Minimum Number Alive for *Arvicanthis dembeensis, Mastomys erythroleucus* and *Tatera robusta* in the Grassland and Maize field CMR-grids at Koka. Columns show monthly rainfall.

The CMR-data show seasonal breeding in *A. dembeensis* and *M. erythroleucus,* less so in *T. robusta* (Fig.2). Females of *A.dembeensis* and *M. erythroleucus* are pregnant or lactating late in the rainy season and the early dry season. The removal trapping data show

similar seasonality in the maize grids but not in the grassland where, throughout the year, nearly every month a small proportion of the females is pregnant (Fig. 3).

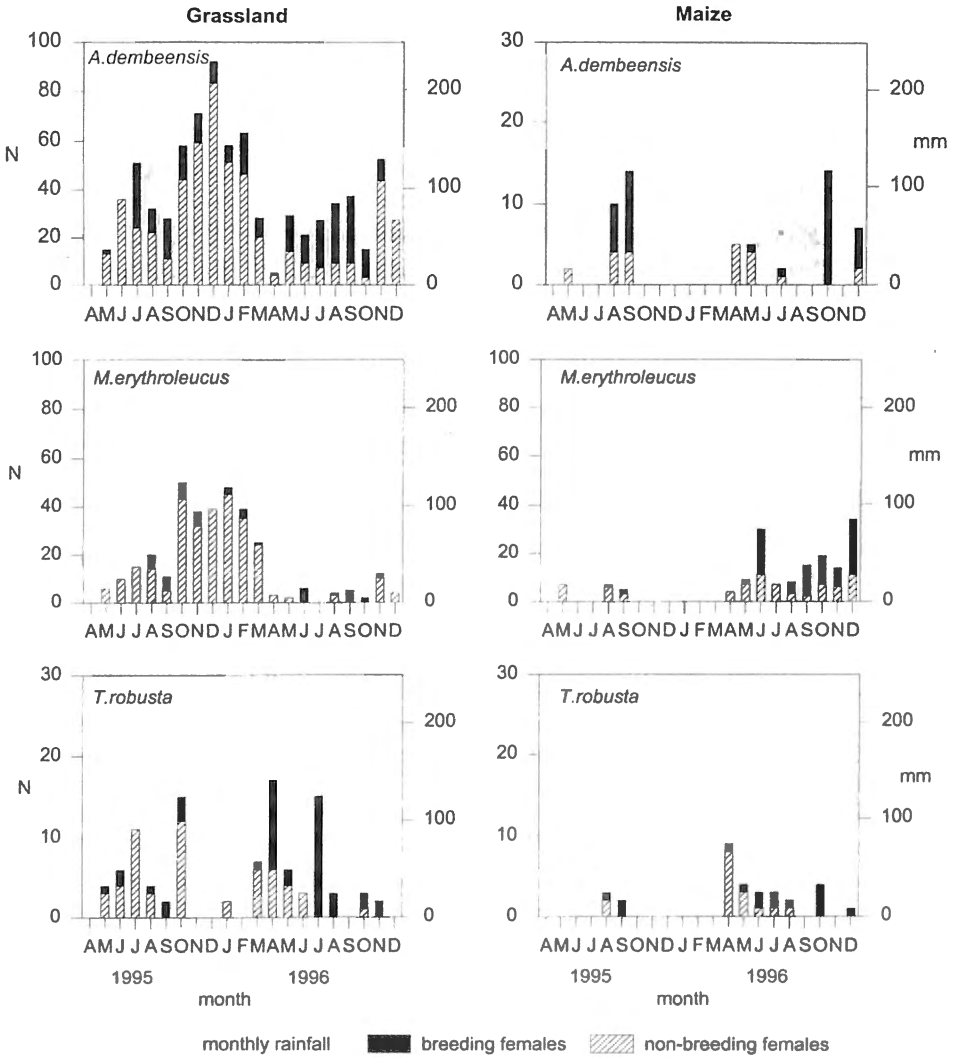

Fig. 2. – Monthly numbers of breeding (lactating or pregnant) and non-breeding females of *Arvicanthis dembeensis, Mastomys erythroleucus* and *Tatera robusta* in the Grassland and Maize field CMR-grids at Koka. Gray background columns show monthly rainfall.

Litter size (number of embryos *in utero*) is also fluctuating seasonally, with higher average values during the second half of the rainy season, both in *A. dembeensis* and *M. erythroleucus*; data for *T. robusta* were too scanty to investigate seasonal patterns (Fig. 4). Overall, litters were smaller in the grassland than in the maize fields for *A. dembeensis*) and *M. erythroleucus* but not in *T. robusta* (Table 2).

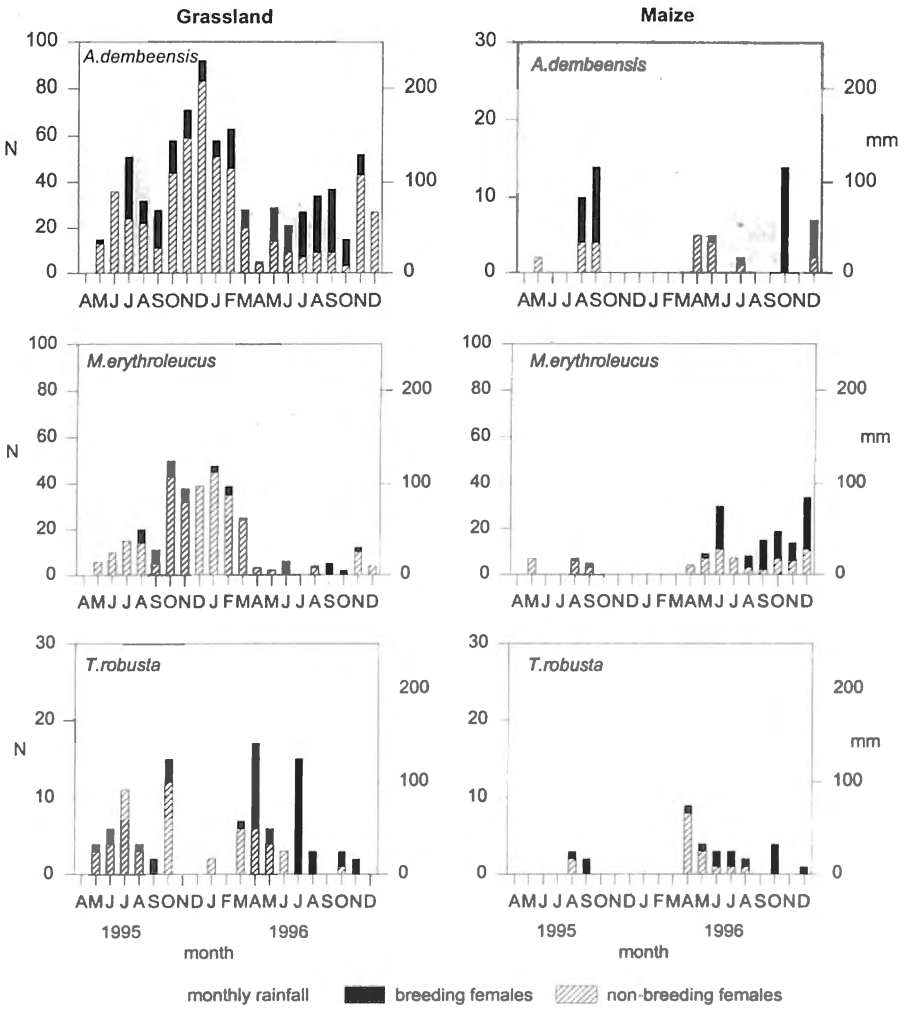

Fig. 3. – Monthly numbers of breeding (lactating or pregnant) and non-breeding females of *Arvicanthis dembeensis, Mastomys erythroleucus* and *Tatera robusta* in the Grassland and Maize removal trapping sites at Koka. Gray background columns show monthly rainfall.

TABLE 2

Litter size for different species in different habitats at Koka and significance of difference between habitats (t-test), (n= sample size)

Species	*Grassland*		*Maize field*		
	mean ± s.d.	*n*	*mean ± s.d.*	*n*	*t-test*
Arvicanthis dembeensis	5.74 ± 2.65	206	7.42±2.63	33	p<0.001
Mastomys erythroleucus	10.21 ± 4.32	32	12.84±3.26	64	p=0.001
Tatera robusta	5.00 ± 1.63	13	5.73±1.72	11	p=0.243

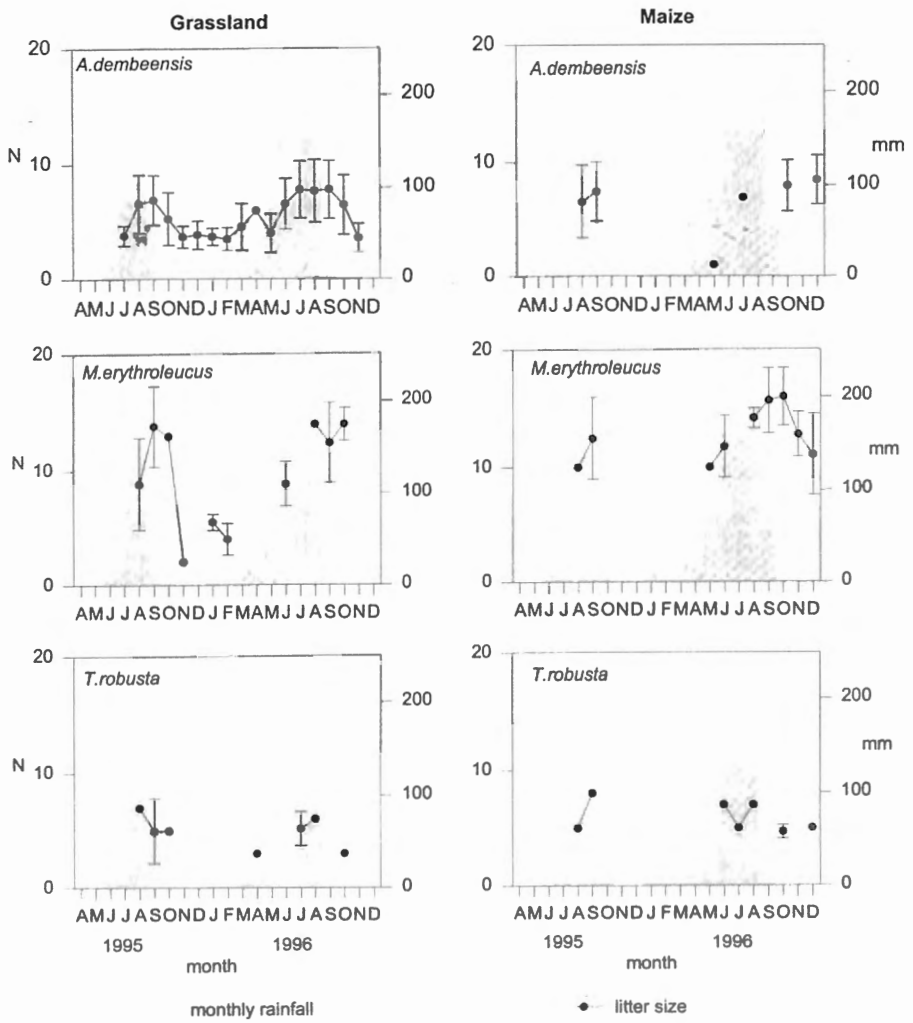

Fig. 4. – Monthly litter sizes (mean ± st.dev) in pregnant females of *Arvicanthis dembeensis, Mastomys erythroleucus* and *Tatera robusta* in the Grassland and Maize field removal trapping sites at Koka. Gray background columns show monthly rainfall.

DISCUSSION

In general, we experienced very low densities of rodents. This was also the case for the two pest species *A. dembeensis* and *M. eryttroleucus* which were nevertheless the most common species in our study and on which we will focus in this discussion. LEIRS (1995) reviewed reported population size estimates for different *Mastomys* species in Africa and found generally much higher fluctuations with maxima of up to a thousand animals per hectare in outbreak years and, more typically, several hundreds during usual seasonal peaks. However, in *M. erythroleucus* in dry Sahel woodland, densities were only around

90 animals per hectare during an outbreak in 1975 and stayed below 10 ind/ha during most other years (HUBERT & ADAM, 1983); similar densities were also reported for *Arvicanthis niloticus* (Desmarest, 1822), which is sometimes considered to include *A. dembeensis* (MUSSER & CARLETON, 1993), during the same outbreak in Senegal (POULET & POUPON, 1978). In a study in Kenya, *A. niloticus* reached higher densities in bushy scrubland but not in grassland (DELANY & ROBERTS, 1978). Thus, although the densities observed in our study are considerably lower than those seen with field mice in Tanzania (LEIRS *et al.*, 1996), they are not atypical. Although our data set is too short for comparisons between years, the literature cited here suggests that, in the grassland, the densities in 1995 were normal, while the increase of numbers in late 1996 could be considered as a small outbreak, at least in *A. dembeensis* and to some extent also in *M. erythroleucus*. Seasonal patterns in density variations were weak, although in both years, there was an increase towards the end of the rainy season; in 1995, however, densities decreased again immediately, while in 1996, the increase started earlier and continued for some months.

In the maize field, densities remained low all the time and there were no clear patterns; also the «outbreak in late 1996» was not seen in the maize fields. Generally lower rodent densities in maize fields, even at peak numbers in grassland, were also observed by GOODYEAR (1976) in the south Central Plateau in Ethiopia. Agricultural activities may play a role in this since the peak densities in the grassland in our study occurred just after the fields were harvested and became, presumably, unfavourable to large rodent populations.

Breeding activity was clearly seasonal in *A. dembeensis* and *M. erythroleucus* in our CMR-study with breeding females in the second half of the rainy season and the early part of the dry season. This is consistent with literature findings from populations of these or related species elsewhere in Africa were breeding also commences a few months after the onset of the rains (*e.g.* DELANY & ROBERTS, 1978; NEAL, 1981; HUBERT & ADAM, 1983; DELANY & MONRO, 1986; LEIRS, 1995 and other references therein). In our removal study, the seasonal pattern was not so clear in the grassland: there, we found breeding females nearly every month. However, our range of fallow-grassland trapping sites was more diverse than that of maize field sites and unsuspected habitat effects may have masked seasonal effects here. In other removal trapping studies, *Arvicanthis* species also showed a less outspoken reproductive seasonality, particularly when habitats were less variable seasonally (DELANY & NEAL, 1969; NEAL, 1981). Obviously, any habitat effects are not visible in a CMR-study on a single, homogeneous site.

Litter sizes in the present study were on the upper side of the published ranges for these species (NEAL 1981; LEIRS, 1995). The seasonal peaks in litter size coincide with the peaks of breeding activity in females in the rainy season and the early dry season. In 1996, when the rains were more abundant than in 1995, the peak of litter size was maintained for a longer period than in the previous year and also the period of intensive breeding activity in females lasted longer. This can explain the higher densities at the end of 1996. The fact that *A. dembeensis* reached higher numbers than *M. erythroleucus*, despite the higher litter size in the latter, may be due to the fact that *Arvicanthis* species have young that mature within a few weeks, while it takes at least two months in *Mastomys* species (DELANY & MONRO, 1985; LEIRS, 1995). This means that *A. dembeensis* young born early

in the breeding season in 1996, could probably still participate in breeding in the same year. This relation between rainfall, particularly the length of the rainy season, and reproductive performance is central in the forecasting model that we intend to apply on our data (LEIRS *et al.*, 1996). Although we need to continue the analysis of our data with respect to maturation and survival, it seems that this model may also be of value to the Ethiopian situation. In order to investigate interannual variation, the field studies at Koka are being continued.

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AN EARLY WARNING SYSTEM FOR IPM-BASED RODENT CONTROL IN SMALLHOLDER FARMING SYSTEMS IN TANZANIA

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Abstract. We conducted a four-year study in Tanzania to test a method for predicting outbreaks of *Mastomys natalensis* rats and verify whether such method, based on rainfall variability, could be used in an Integrated Pest Management strategy for rodent control. Temporal fluctuations in rodent numbers and breeding activity were monitored at four localities with different rainfall regimes. Breeding peaked towards the end of the main rainy season and continued into the dry period. When the short rains of October-January were unusually abundant and well distributed, reproduction started earlier and rodent numbers increased faster. Where abundant short rains were a normal condition returning every year, such effect was not clear. A method to assess rodent damage to germinating seedlings was found to be robust and can be used for monitoring rodent problems. Using this assessment technique, we showed that the effects of a single control action undertaken at planting time do not persist long enough to protect seedlings, probably due to quick reinvasion of the treated fields by rodents from the surroundings. These observations are formulated into a rodent control package whose steps are to predict rodent outbreaks, to warn farmers and the government of the outbreaks, and to organise control measures in advance.

Key words: IPM, *Mastomys natalensis*, crop damage, rodent outbreaks, forecasting, early warning, Tanzania.

INTRODUCTION

Field rodents belonging to species of the genus *Mastomys* are among the major pests that affect production of food cereals in many sub-Saharan countries. The multimammate rat *Mastomys natalensis* (Smith, 1834) is the most important rodent pest in Tanzania. At planting time this rat digs out planted seeds and germinated seedlings. Populations of *M. natalensis* undergo irregular population explosions with densities as high as 1000 rats per hectare; the outbreaks occur during the dry season and last through the planting period of October-February (TELFORD, 1989; MWANJABE & SIRIMA, 1993). Rodent control on fields then becomes a necessity. The majority of farmers in Tanzania are smallholders, owning 0.5-2 ha of fields per household. The fields are usually isolated plots surrounded by bushes and fallow fields (MWANJABE, 1993). At a low rodent infestation, crop protection by individual farmers is quite feasible. In times of outbreaks, however, rodent control measures have to be on a large scale and organised, or at least facilitated, by the government. In order to enable the government to plan effective control operations far in advance

of the outbreaks, an alert system should be implemented (TAYLOR, 1968; SHUYLER, 1977; MWANJABE, 1990).

TELFORD (1989) and LEIRS *et al.* (1989) reported that reproduction in a population of *M.natalensis* in Morogoro, Tanzania, was strongly linked to rainfall and suggested that unusual rainfall may initiate aseasonal breeding resulting in higher densities. This idea, and the underlying life history particulars, were further investigated and this resulted in an outbreak forecasting model, based on the occurrence of unusually high rainfall during the first part of the rainy season (LEIRS *et al.*, 1996). In the present study we investigate to what extent this forecasting model would be applicable in other localities in Tanzania with different rainfall regimes and how it can be incorporated into an integrated pest management (IPM) package.

MATERIAL AND METHODS

Rodent population studies

Four study sites were selected (Table 1). Site 1 is the same site where the forecasting model to be tested was developed (LEIRS *et al.*, 1996). Sites 1, 2 and 4 have a bimodal rainy season with so-called short rains (in Swahili «*vuli*») at the end of the year and long rains (in Swahili «*masika*») between February and May. Although rainfall is variable between years, the long rains are predictably abundant at these three sites, while the short rains are in most years, but not always, rather poor in sites 1 and 2; site 3 has reliably good short rains allowing a *vuli* planting season every year. Site 4 has a unimodal rainy season between November and April, peaking early in the year.

TABLE 1

Information on the four study sites

	Site 1 *Morogoro*	*Site 2* *Makuyu*	*Site 3* *Chunya*	*Site 4* *Kibwaya*
	06° 51¢S, 37° 38¢E	06° 22¢S , 37° 38¢E	08° 46¢S, 33° 18¢E	06° 57¢S, 37° 49¢E
Trapping				
Study period	Jan'93-Sep'86	Apr'93-Aug'96	Jun'94-Dec'96	Jul'94-Aug'96
No. of sessions	33	24	12	12
Trap nights	18976	13500	8388	7484
Rainfall				
Short rains	Nov-Jan	Nov-Jan	-	Nov-Jan
Long rains	Feb-May	Feb-May	Dec-Apr	Feb-May
Farming activities				
Planting:				
short rains	-	Nov.	-	Nov
long rains	Feb-Mar	Feb	Dec-Jan	Feb
Harvesting	Jul-Sep	Aug	Jul-Sep	Jul-Sep

In each site, we selected maize field areas where we collected animals by removal trapping using metal snap traps, following the small quadrat method (MYLLYMÄKI *et al.*, 1971). In each trapping session, 15-20 small quadrats (15 m x 15 m) were set with 12 traps each, depending on the number of available traps. Total number of trap nights set for each site are shown in Table 1. Traps were baited with dry sardine fish and coconut and left overnight for three consecutive nights per sampling session. Sampling sessions were organised monthly at sites 1 and 2 but rather irregularly at the other, less accessible, sites (Table 1). Where trapping was undertaken every month, the actual trapping fields were changed monthly.

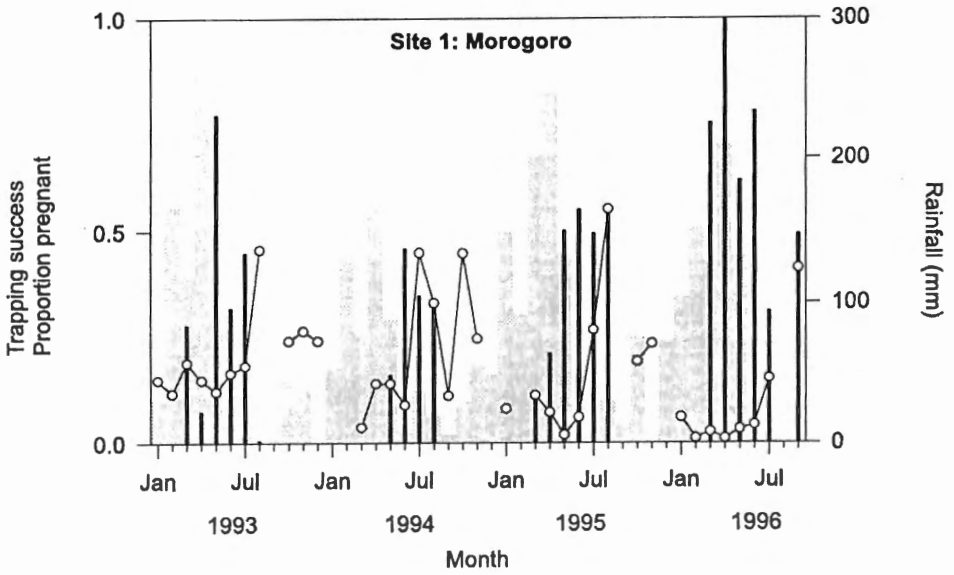
For each captured animal we recorded body weight, head and body length, testis length, and pregnancy (presence of embryos at autopsy). Population size was expressed as trap success index, *i.e.* number of captured animals per trap nights, adjusted for trap saturation (CAUGHLEY, 1977). A breeding index (percentage of pregnant females) was used to recognize reproductive periods. Rainfall data were obtained from national meteorological stations and from rain gauges installed in the research sites.

Crop damage assessment

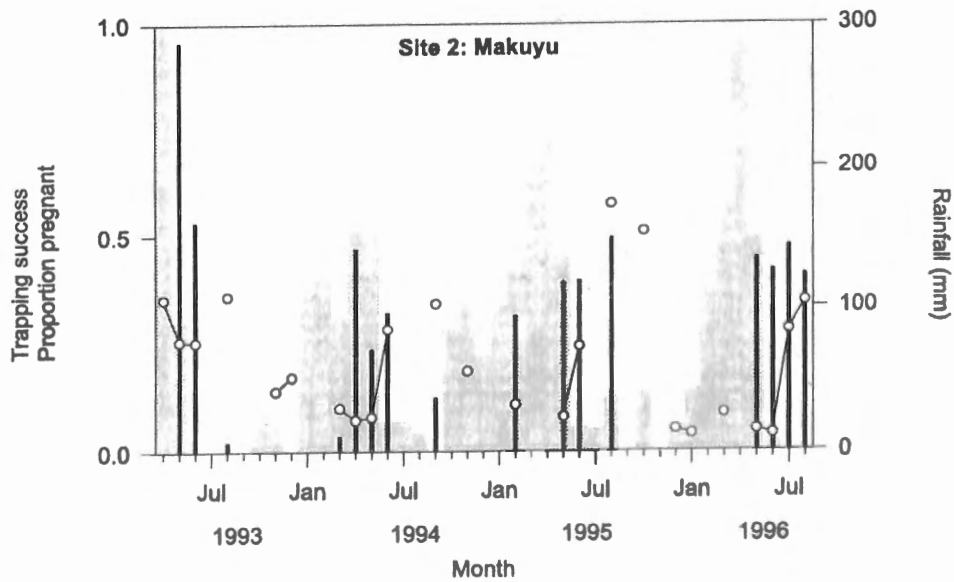
At planting time, we carried out damage assessment surveys in farmers' fields at different sites. The method at planting time was based on the one used in South East Asia where rat-cut tillers of paddy are counted at each hill (BUCKLE, 1994). In Tanzania, however, rats start retrieving cereal seeds immediately after sowing. In such a situation it would be difficult to count seeds removed by rats before emergence. Therefore, we counted missing (= unemerged) seedlings plus those actually found cut by rats after emergence. Before planting, we made arrangements with the farmers to plant a fixed number of three seeds per planting hole. The sampling units were maize rows, five rows apart, leaving out two edge rows all round the field. We sampled ten rows in fields of less than 1ha and twenty rows in larger fields. The assessor walked along maize rows across the field, counting missing seedlings at each hole or stand. Damage ($D\%$) was calculated as $D=100d/(u+d)$, with d being the total number of missing seedlings in the whole sample; and u the number of undamaged seedlings. Before the harvest at site 2, we also assessed damage on gnawed maize ears. A maize plant in a stand was considered damaged if 25% of its ear was found gnawed by rats. Estimation of crop loss was calculated using the same formula as above.

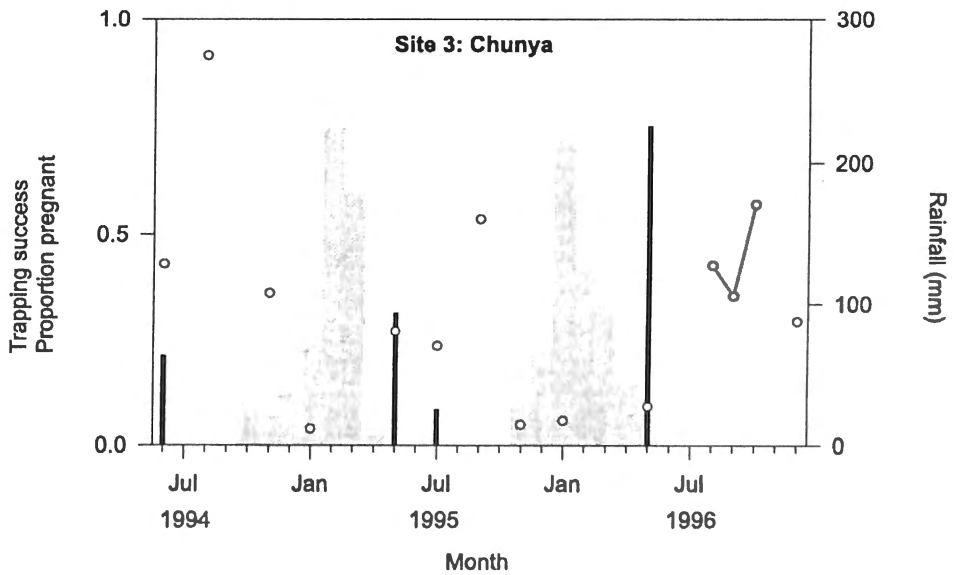
Recolonisation of treated fields

In order to investigate to what extent quick recolonisation of vacant fields would minimise the effects of rodenticide application at the time of planting, we set up an experiment at site 1. Four 0.5 ha maize field plots were selected, each one surrounded by fallow land; a rodent control action was undertaken in two of the plots, at the same day the seeds were planted. We applied a rodenticide bait with 1.5% zincphosphide, laid out in the field in small heaps of approx. 20 g, 10 m apart. A 10 m-wide margin around each treated plot was also baited. Dead animals found the next morning and autopsied confirmed the efficacy of the poisoned bait. Damage assessments were carried out for each plot as described above one and two weeks after planting; later on, young maize plants are no longer at risk from rodent damage. The changing abundance of rodents during this experiment is presented elsewhere (LEIRS *et al.*, 1997).

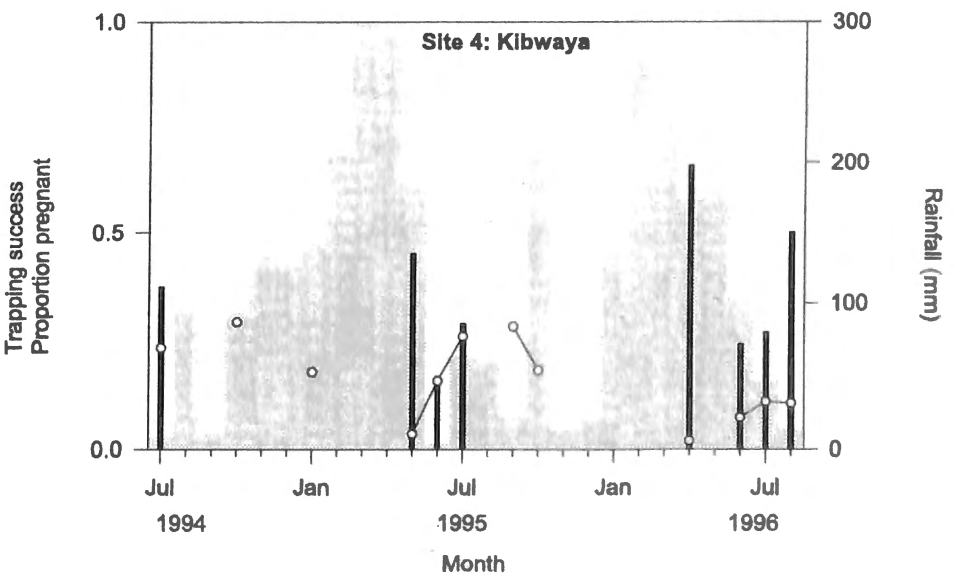


Figs. 1-2. – Monthly rainfall (grey columns), trapping success index (circles) and proportion of pregnant females (narrow black columns) at the four study sites. There were no captures in months for which no trapping success index is indicated.





Figs. 3-4. – Monthly rainfall (grey columns), trapping success index (circles) and proportion of pregnant females (narrow black columns) at the four study sites. There were no captures in months for which no trapping success index is indicated.



RESULTS

Data of rodent abundance and breeding activity were plotted together with monthly rainfall for each of the four study sites (Figs. 1-4). Rainfall during the study period was typical for each of the sites with short unimodal rainy seasons at site 3 and longer seasons at the other sites. Sites 1 and 2 had poor *vuli*-rains at the end of 1993 and 1995 but more rain at the end of 1994-start of 1995. At site 4, rainfall was also abundant and evenly spread in late 1994 but in late 1995 it was concentrated in October and November and December were dry. No rodent population outbreaks occurred during the study, but trapping success was high at site 3 in mid 1994. Trapping success varied considerably seasonally with, at all sites, low values during the rainy seasons, increasing towards the end of the rains and peaking well into the dry season. Breeding was also strictly seasonal with pregnant females appearing in the population about 1-2 months after heavy rains had occurred. When there was more rain in the first part of the rainy season, breeding started earlier (Figs. 1 & 2). At site 4, this could not be observed directly since there were no data for the first months of each year (Fig.4). However, in May 1995 there were many young and subadults in the population (10/23 animals weighed less than 30 g) while in the same month in 1996, there were only adults (0/14 animals <30g), suggesting earlier breeding in 1995, following the wet end of 1994. At site 3 rainfall was not very different between years and we have no indications for different breeding patterns neither (Fig. 4).

TABLE 2

Damage estimates at sites 2 and 4 shortly after planting and before harvest. All estimates are replicated for several plots at each site

Site	*Plot size*	*Post-planting*		*Pre-harvest*	
		Date	*Damage %*	*Date*	*Damage %*
2	0.8	20 Dec. 1993	70.4	4 July 1994	8.1
	0.4	"	77.2	"	6.6
	0.4	"	76.9	"	8.8
	0.4	"	74.9	"	9.3
	0.8	"	88.6	"	13.3
	0.8	"	82.3	"	10.6
4	0.5	4 Jan. 1994	49.21		
	0.5	6 Jan. 1994	38.51		
	0.5	26 Jan. 1994	43.43		
	0.5	1 Feb. 1994	39.58		

Damage assessments were easily performed with the chosen method. Damage figures were high, up to more than 80 % of the seedlings were damaged at one site. We obtained similar estimates in different plots at a same site but important differences between sites (Tables 2, 3).

TABLE 3

Damage assessment in maize seedlings in Zn_1P_3-treated and untreated control plots at Site 1. Planting and treatment were done simultaneously on 6 March 1995. Damage assessments were made one week and two weeks after planting

Field	*after 1 week*	*after 2 weeks*
Treated 1	42.51 %	43.85 %
Treated 2	40.78 %	39.12 %
Control 1	38.51 %	48.68 %
Control 2	47.03 %	58.05 %

In the recolonization experiment, seedling damage estimates were high in both treated and untreated fields (Table 3). After one week, there was no difference in damage between both field types (t-test, $p=0.82$), but after two weeks, the damage had increased in the control fields (paired t-test, $p=0.03$) but not in the treated fields ($p=0.93$); the difference between fields, however, remained small and non-significant (t-test, $p=0.15$)

DISCUSSION

The observed patterns of rainfall and breeding confirm what has been documented before for populations of *M. natalensis* in Tanzania (CHAPMAN *et al.*,1959; TELFORD, 1989; LEIRS *et al.*, 1989). Breeding starts soon after heavy rains and continues into the dry season. This is the case regardless of whether the rainy season is unimodal or bimodal. However, at site 4, where the short rains were very abundant in late 1994, there was not yet evidence of reproduction three months after the start of the rains, although we have indirect indications from the weight distributions in May for that site that breeding in 1995 did effectively start earlier than in 1996. This means that the basic biological mechanism of the forecasting model suggested by LEIRS *et al.* (1996), i.e. the relation between rainfall and breeding season, holds as a general rule in Tanzania. Such was also clear in many other studies elsewhere in Africa (reviewed in LEIRS, 1995). The present study is less conclusive on the importance of the short rains in determing population dynamics but this is due to unfortunate gaps in the trapping series and the absence of aseasonal rainfall during our study period at site 3. At least in the areas with bimodal rainy seasons and usually poor short rains, the hypothesis holds.The early breeding, if any, in the area with usually abundant short rains (site 4), was slower and this means that the population dynamics effects due to the quick succession of generations (LEIRS *et al.*, 1993) will be smaller. It is worth noting that FRENCH (1975) already concluded from a simple mathematical model that conditions with two clearly distinct rainfall peaks cause larger populations of *Mastomys* sp. than a single extended rainy season. Under the latter conditions, the validity of the forecasting model proposed by LEIRS *et al.* (1996) is not obvious.

The damage assessment technique that we used shortly after planting can be considered to be robust, judging from the very similar results obtained in different plots at the same site. Since the technique is also easy to apply, it can be used as a monitoring tool for

IPM-strategies. The absolute accuracy of this method relies on the assumptions that the seed has high germinating viability, and that there is enough soil moisture in the soil to allow germination. Under complete dry soil conditions, seeds remain in the ground intact until the rain comes but in partially moist soil, germination may be impaired due to seed moulding. Another assumption is that no pests other than rodents attack the seed. Insect or disease damage is of no big concern for the technique since there are many typical characteristics of rodent damage (digging activity, gnawing traces). Birds are probably incapable of retrieving seeds that are sown deep enough and well covered with soil (ANONYMOUS, 1987). These assumptions make that the damage assessments obtained in different years and at different sites cannot easily be compared. Clearly, estimates obtained after planting are of a different nature than those obtained before harvest and therefore they cannot be combined.

The observed damages after planting were high. During a study conducted in Chunya (Site 3 in our study) and other parts of Tanzania, a 10% damage of maize seedlings resulted in a 9.9% crop loss at harvest (MYLLYMÄKI, 1989). The damage levels of 40% to 80% that we observed in sites 1, 2 and 4 could thus be expected to cause serious losses of food crop. Farmers use informal damage assessments after planting to decide whether and how much they should replant. A more formal recording of damage assessments could be used to follow up and adjust predictive models and if they can be linked by future studies to rodent abundances, they can also be used as input for such models.

The used technique of damage assessment after planting seems also valuable to assess the effects of pest control applications. This was obvious in our recolonization experiment, where damage assessment showed rodent problems both in the treated and untreated fields. This corresponds to the observations that were made on rodent abundance during this same experiments (LEIRS *et al.*, 1997). Briefly, they found a quick decrease of rodent abundance immediately after the ploughing and planting, intensified of course by the rodenticide application in the treated fields: after a few days, densities increased again sharply and became even higher than before the planting, both in the treated and untreated fields. Clearly, our experimental fields were too small for the control effect to persist during a longer period. In terms of IPM strategies, these observations imply that rodent control operations should be started shortly before planting starts, and continued until at least one week after emergence of the crops (i.e. when the crops are no longer at risk from rats). At pre-harvest, use of rodenticides would be less effective because by then bait would be competing with rich food sources in the crop fields themselves. The most effective way to prevent crop loss at this time is to harvest the crop as soon as it is ready for harvest.

Our study showed that the presumed predictors of rodent outbreaks, abundant short rains and off-season breeding, may be of more general importance than proven until now. The predictive accuracy of a system based on this relation will depend on the number and distribution of monitoring sites in the country. A network of meteorological stations is present in Tanzania so that reliable rainfall data can be used. Surveys of rodent breeding and populations require specialist staff and equipment but are only needed for actual research; the monitoring of rodent problems can be based on simple damage assessments. The governments at different levels should be prepared to act accordingly when an outbreak

forecast is issued and allow easy access to rodent control means. After the abundant short rains in late 1994, we issued a rodent outbreak warning to the Ministry of Agriculture but no action was undertaken; in 1995, farmers in southern Tanzania complained about unusually high rodent damage but unfortunately, extension services were not prepared for the situation.

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EXTRACTION OF NUTRIENTS FROM *PROTEA* POLLEN BY AFRICAN RODENTS

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Abstract. Many of the species of *Protea* that are found in the south-western Cape of South Africa are pollinated by rodents. In Australia, where flowers of the same family are also mammal-pollinated, some of the mammal species that feed on Proteaceae flowers not only gain energy from nectar but also extract protein from pollen. This contrasts with the widely held belief that most mammals are unable to extract nutrients from pollen. To determine whether African rodents are also capable of using pollen as a source of protein, faecal samples were collected from mammals trapped at two sites in the Western Cape where *Protea humiflora* and *P. subulifolia* were common. The mammals included three rodent species, *Rhabdomys pumilio*, *Aethomys namaquensis* and *Mus minutoides*, and an elephant shrew, *Elephantulus edwardsii*. The mean percentage of empty or partially digested pollen grains was 50.3% for *E. edwardsii*, 56.8% for *R. pumilio*, 60.4% for *A. namaquensis* and 83.0% for *M. minutoides*. These four species are clearly capable of penetrating the pollen grains of *Protea* during digestion. Pollen is therefore a potential protein source for these species.

Key words: pollen, rodent, diet, protein, nitrogen.

INTRODUCTION

Many mammalian species, including rodents, feed on flowers or flower products. For example, in Britain, the dormouse, *Muscardinus avellanarius* (L.), feeds on the anthers of hawthorn, *Crataegus monogyna*, in early spring (RICHARDS *et al.*, 1984) and, in the Kalahari desert, *Acacia* flowers are eaten in large quantities by larger mammals such as springbok, *Antidorcas marsupialis* (Zimmerman, 1780), and giraffe, *Giraffa camelopardalis* (L.) (SAUER, 1983; NAGY & KNIGHT, 1994). Although in many cases the plants receive no corresponding benefit, a range of mammal species including bats, rodents, marsupials, primates and insectivores are involved in the pollination of various plant species (REBELO & BREYTENBACH, 1987; GOLDINGAY *et al.*, 1991; FERRARI & STRIER, 1992; FLEMING, 1993).

What do flowers have to offer rodents? One flower product that rodents are likely to feed on is nectar. Nectar is a sugar solution produced by many flowers to attract pollinators and it should provide foraging rodents with an easily obtainable source of energy. A second flower product that rodents may feed on is pollen. Pollen can have a very high protein content. The pollen of some mammal-pollinated *Banksia* species contains over 30% crude protein (TURNER, 1984). The bulk of the protein in a pollen grain is found in the cell

contents, known as the protoplast, and this protoplast is encased within a hard cell wall that is extremely resistant to chemical breakdown (RAVEN *et al.*, 1992). The strength of this cell wall has led to a belief that it is difficult for small mammals to extract nutrients from pollen (HUME, 1982). Some dietary studies have identified pollen as a major component of faecal samples but discounted it as a possible source of nutrition because of this perceived difficulty (*e.g.* SMITH, 1982).

Evidence that pollen may not be such an inaccessible food source has been found in Australia in recent years. Laboratory studies on two flower-feeding marsupials, *Petaurus breviceps* (Waterhouse, 1838) and *Cercartetus nanus* (Desmarest, 1818), showed that *Eucalyptus* pollen had a high biological value for both species. Furthermore, the maintenance nitrogen requirements for both species were exceptionally low when they were fed diets in which pollen was the only source of nitrogen (SMITH & GREEN, 1987; VAN TETS, 1996). A flower-feeding bat, *Syconycteris australis* (Peters, 1867), has also been found to have a low maintenance nitrogen requirement on pollen, although not as low as for the marsupials (LAW, 1992a).

A large proportion of the *Banksia* pollen grains found in faecal samples taken from small Australian mammal species have been empty, indicating that these species are capable of extracting the protoplast from the *Banksia* pollen grains they ingest. In the faeces of the obligate flower-feeding marsupial *Tarsipes rostratus,* 95-100% of the *Banksia* pollen grains were empty (RICHARDSON *et al.*, 1986). Other marsupials that frequently fed on flowers, such as *P. breviceps* and *C. nanus,* removed the protoplast from approximately 65% of the pollen grains (VAN TETS & WHELAN, 1997). The flower feeding bat, *S. australis*, was able to extract the protoplast from 53% of the *Banksia* pollen grains it ingested (LAW, 1992b). Even mammals for whom flower products were unlikely to form an important part of their diet were able to digest *Banksia* pollen. For example, the rodent *Rattus fuscipes* (Waterhouse, 1839) and the insectivorous marsupial *Antechinus stuartii* (Macleay, 1841) removed the protoplast from 55% and 37% respectively of the pollen they ingested (VAN TETS & WHELAN, 1997).

In the Cape Floral Kingdom of south-western South Africa, rodents regularly visit the inflorescences of *Protea* in search of food, and rodents are the primary pollinators of a number of *Protea* species (WIENS *et al.,* 1983; REBELO & BREYTENBACH, 1987). These species produce large and often cryptic inflorescences close to ground level (REBELO & BREYTENBACH, 1987). The inflorescences have a strong musky odour and they release nectar at night with maximum flower opening corresponding to maximum small mammal activity (WIENS *et al.,* 1983). When small mammals were excluded from the inflorescences of two species, *P. humiflora* and *P. amplexicaulis*, seed set was reduced by 50 and 95% respectively (WIENS *et al.,* 1983).

Protea and *Banksia* both belong to the same family, Proteaceae. Many relevant species of *Protea* flower between mid-winter and mid-spring (REBELO, 1995). As this is a period when other food resources are in short supply, it is possible that the flower products of *Protea*, including pollen, are an important element in the diet of the rodents during those periods. Although, there is no evidence that the rodents actively select pollen while foraging, they do ingest large quantities of pollen while grooming (WIENS *et al.,* 1983). As Australian mammals, including the rodent *Rattus fuscipes*, could extract the protoplasts

from the pollen grains of plants from the same family, it was likely that at least some of the South African rodents could do the same with *Protea*. My aim, therefore, was to determine whether the rodents involved in the pollination of *Protea* were removing the protoplasts from the pollen they ingested.

MATERIAL AND METHODS

Between 31 July and 2 August 1996 and between 17 and 20 September 1996, small mammals were captured at two sites in which the inflorescences of rodent-pollinated species of *Protea* were abundant. The first site was on the foothills of Jonaskop on the western edge of the Riviersonderendberge (33° 56'S 19° 31'E) in which *Protea humiflora* was the only species of *Protea* in flower at the time of sampling. The other site was near Kaaimansgat in the Stettynsberge (33° 56'S 19° 17'E). At Kaaimansgat, *P. subulifolia* was the most common species of *Protea* within the site but there were a few *P. laurifolia* plants in flower near its edge. The mammals were captured in live traps baited with peanut butter, oats and golden syrup and were toe-clipped so that samples were taken from each individual only once. Faeces were taken from the traps in which the small mammals were caught and were stored in 70% ethanol.

Faecal samples were taken from two species of mammal at Jonaskop: the Namaqua rock mouse, *Aethomys namaquensis* (A. Smith, 1834), and Edward's elephant-shrew, *Elephantulus edwardsii* (A. Smith, 1839). At Kaaimansgat, samples were taken from two different species: the striped field mouse, *Rhabdomys pumilio* (Sparrmann, 1784), and the pygmy mouse, *Mus minutoides* (A. Smith, 1834). A number of other species, including

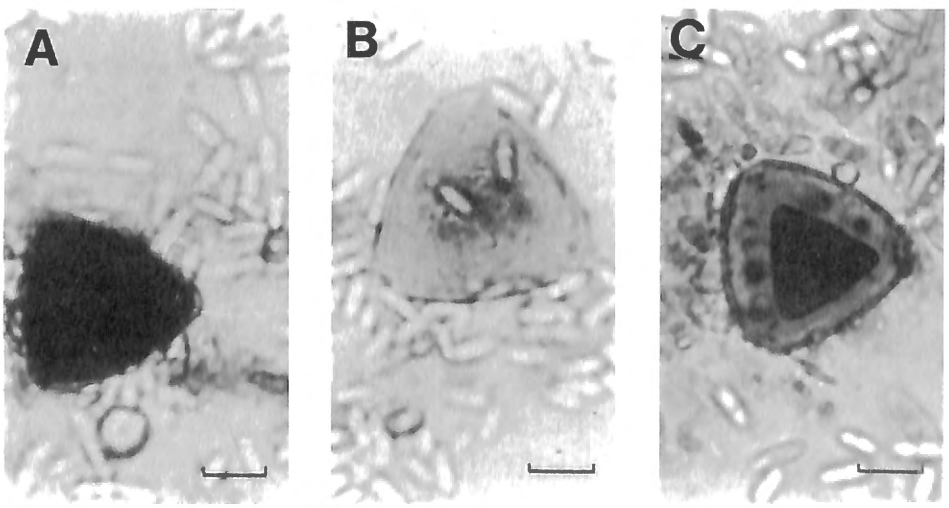

Fig. 1. – *Protea humiflora* pollen grains in the faeces of *Aethomys namaquensis*. A is an intact pollen grain. The darkly stained protoplast fills the entire cell. B is an empty pollen grain. Only the cell wall is visible. C is a partially digested pollen grain, the stained trianglular shaped structure in the centre of the cell is the remnant of the protoplast. The scale bars represent 10 mm.

Acomys subspinosus (Waterhouse, 1838), *Otomys irroratus* (Brants, 1827) and *Myosorex varius* (Smuts, 1832), were also captured at the two sites. These were not included in this study as fewer than five individuals were captured from each of these species.

Approximately 10 mg of faeces from each animal was spread on a microscope slide and stained with a drop of cotton-blue lactophenol. This stained the protoplast dark blue but left the cell wall unstained. On each slide, 100 *Protea* pollen grains were counted and the percentage of grains from which the protoplast had been removed, even if only partially, was recorded (Fig. 1). Grains with partially digested protoplasts were included with the completely empty grains, as in both cases the pollen grain had been penetrated during its passage through the digestive tract. Samples of pollen were also taken directly from the pollen presenters of *P. humiflora* and *P. subulifolia* and assessed in a similar manner. The percentage of empty and partially digested pollen grains in the faeces of the four species was compared using a one way analysis of variance. The values were transformed using an arcsine transformation prior to the analysis.

RESULTS

Over 99% of the pollen grains taken directly from the flowers were intact. However, on average, over half the *Protea* pollen in the faeces of all four species were either empty or partially digested (Fig. 2). The mean percentages were 49.0% for *E. edwardsii*, 58.4% for *R. pumilio*, 60.4% for *A. namaquensis* and 83.0% for *Mus minutoides*. There was no significant difference between the values for *E. edwardsii*, *R. pumilio* and *A. namaquensis*. However, the mean percentage of empty or partially digested grains for *Mus minutoides* was significantly higher than for the other three species ($P < 0.05$).

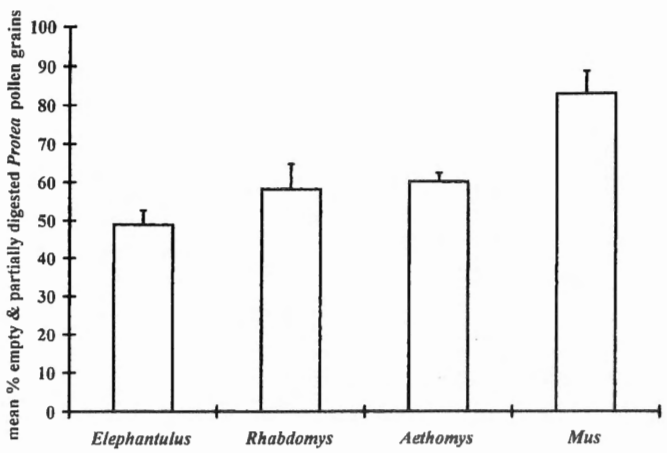

Fig. 2. – Mean percentage of penetrated *Protea* pollen grains in the faeces of four small African mammal species. Error bars represent standard errors. N = 10 for *Elephantulus edwardsii*, 8 for *Rhabdomys pumilio*, 8 for *Aethomys namaquensis* and 5 for *Mus minutoides*.

DISCUSSION

As all four species are capable of extracting the protoplast from at least half the *Protea* pollen grains that they ingest, the pollen cell wall does not prevent them from gaining access to the protein of most of the *Protea* pollen that has been ingested. None of these species is a specialist flower feeder. Their ranges all include areas where the genus *Protea* does not occur (SKINNER & SMITHERS, 1990). Therefore, it is likely that most small African rodent species will also be able to digest *Protea* pollen.

The percentages of empty *Protea* grains were very similar to the percentages of empty grains found in the faeces of eutherian mammals feeding on *Banksia* pollen in Australia: 55% for *R. fuscipes* and 53% for *S. australis* (LAW, 1992b; VAN TETS & WHELAN, 1997). This similarity between the digestibility of *Protea* and *Banksia* suggests that similar values could be expected for other Proteaceae pollens ingested by small mammals.

The mechanism used to extract the protoplast is unclear at this stage and a number of mechanisms have been proposed by various researchers. These include the induction of germination, osmotically or chemically induced bursting and direct enzymatic digestion (TURNER, 1984; RICHARDSON *et al.*, 1986). Of these, the direct enzymatic digestion of the protoplast through the pores of the pollen grain seems to be the most likely mechanism in this case, as partially digested grains were present in the samples and pollen tubes and pollen grains that had obviously burst were not observed. If this is the case, then the percentage of empty pollen grains is likely to be an underestimate, as many grains that appeared intact under a light microscope may have been partially digested. This is supported by an earlier study which found that the apparent digestibility of *Eucalyptus* pollen nitrogen for *C. nanus* was higher than the proportion of empty pollen grains in its faeces (VAN TETS, 1996).

The importance of *Protea* pollen relative to other protein sources in the diet of these small mammals is even less clear. It is dependent on a number of variables including the quantity of pollen available, the foraging behaviour of the mammalian species and the ability of that species to absorb and retain the protein contained in the pollen. Unfortunately, very little data is available on any of these variables. However, as the pollen of *Eucalyptus* (the only pollen that has been looked at in detail in this respect) proved to be a very good source of nitrogen for three mammal species (SMITH & GREEN, 1987; LAW, 1992a; VAN TETS, 1996), it is likely that this is also true for *Protea* pollen.

The success of these four species in extracting the protoplasts from the pollen of *Protea*, taken in conjunction with the Australian data for mammals feeding on *Banksia* and *Eucalyptus*, suggests that small mammals may be able to extract nutrients from many pollen species. Pollen is often rich in protein, and in areas where flowers are seasonally abundant rodents may ingest it in large quantities. It should not be overlooked in dietary studies of rodents in such areas.

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POPULATION STRUCTURE AND REPRODUCTIVE CYCLE OF *PRAOMYS JACKSONI* (DEWINTON, 1897) AND FIRST DATA ON THE REPRODUCTION OF *P. MISONNEI* VAN DER STRAETEN & DIETERLEN, 1987 AND *P. MUTONI* VAN DER STRAETEN & DUDU 1990 (MURIDAE) FROM MASAKO FOREST (KISANGANI, ZAIRE)

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Abstract. The three *Praomys* species occuring in Masako forest have a different distribution over the four habitats investigated. *P. jacksoni* is the most common and widespread species whereas *P. misonnei* and *P. mutoni* are less common and restricted to particular habitat types. The population structure of *P. jacksoni* is very similar from year to year with a predominance of adult animals in each month. Reproduction is continuous throughout the year and variations in intensity are more pronounced between years than between seasons. Data on the reproduction of two newly described species, *P. misonnei* and *P. mutoni*, are presented.

Key words: Rodentia, *Praomys*, Zaire, population biology.

INTRODUCTION

During a 3-year study data were collected on the population dynamics and reproduction of rodents from Masako forest near Kisangani (DUDU, 1991). Beside *Praomys jacksoni* (De Winton, 1897) , which is the most common rodent species in this forest, two new species of the genus *Praomys* were described, *P. misonnei* Van der Straeten & Dieterlen, 1987 and *P.mutoni* Van der Straeten & Dudu, 1990. Since no data are yet available on the biology of these newly discovered species we present data on their distribution and reproduction in comparison with that of *P. jacksoni*.

MATERIAL AND METHODS

From December 1984 to November 1986 rodents were collected in the Masako forest using Victor snap traps placed in four different habitats; primary forest, old secondary

forest, fallow land and very wet habitats along rivers which are inundated periodically (Fig. 1).

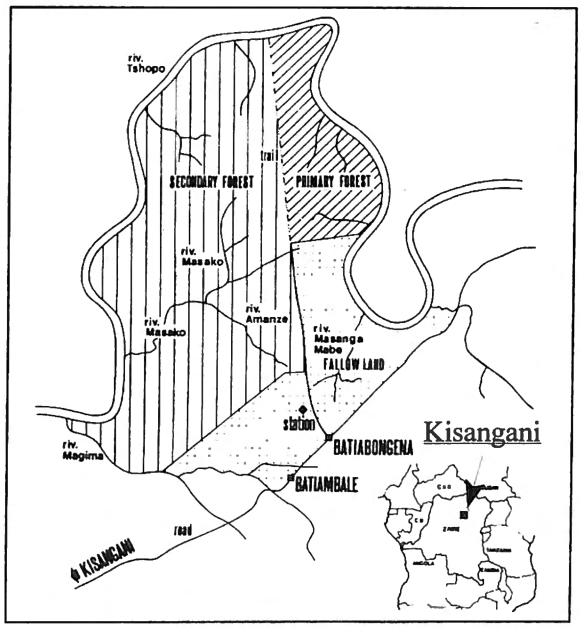

Fig. 1. – Situation map of Masako forest with the different collecting sites.

According to their weight animals were grouped as juveniles, subadults and adults. Because only few individuals were collected of the last two species data were grouped into 3 month periods.

RESULTS

In total 938 *P. jacksoni* (552 males: 386 females), 33 *P. misonnei* (22 males: 11 females) and 34 *P. mutoni* (21 males: 13 females) were captured.

In Table 1 the occurence of the three *Praomys* species in the different habitats is given. None of the species was evenly distributed over the four habitat types (*P. jacksoni* $c2=18.255$, $p=0.0003$; *P. missonei* $c2=9.121$, $p=0.0277$; *P. mutoni* $=194.321$, $p=0.0000$). *P. jacksoni* was the most common of the three *Praomys* species with a preference for fallow land and secondary forest. While *P. misonnei* was captured in all habitat types except riverine habitat, *P. mutoni* seems to occur primarily in this kind of habitat. Captures of *P.mutoni* in the other habitats were always situated close to small rivers. The distribution of the three species over the different habitat types was significantly different (Pearsons $c2=250.9$, $df=6$, $p<0.0000$).

The removal data reveal that the sex ratio of *P. jacksoni* is strongly male biased ($n=938$, $sr=0.59$, $X2=29.4$, $p<0.001$). In *P.misonnei* and *P. mutoni* the sex ratio is also biased in favour of the males although not significantly (resp. $sr=0.67$ and $sr=0.62$).

TABLE 1

Number of captures of the 3 Praomys species within the different habitat types (number of captures per 100 trap nights between parenthesis)

Habitat	*Trap nights*	*P. jacksoni*	*P. missonei*	*P. mutoni*
Primary forest	800	47 (5.9)	4 (0.5)	0 (-)
Secondary forest	6200	466 (7.5)	22 (0.4)	6 (0.1)
River borders	1300	57 (4.4)	0 (-)	27 (2.1)
Fallow land	5400	405 (7.5)	9 (0.2)	1 (0.02)

The weight distribution of the collected *P. jacksoni* indicates that all age groups are equally present throughout the year (Fig. 2).

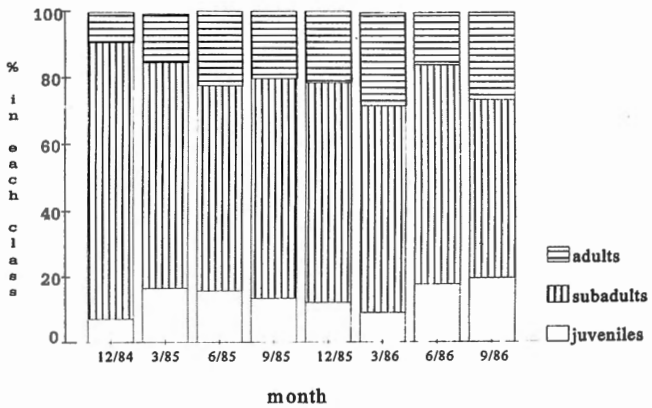

Fig. 2. – Distribution of the different age classes of *Praomys jacksoni.*

The percentage of sexually active adult males (scrotal) and females (perforated, pregnant or lactating) of *P. jacksoni* is presented in Fig. 3. It is clear that there is little or no seasonal variation in reproductive activity whereas differences between years are more pronounced. The few data that are available for the two other species also indicate that reproduction is continuous throughout the year.

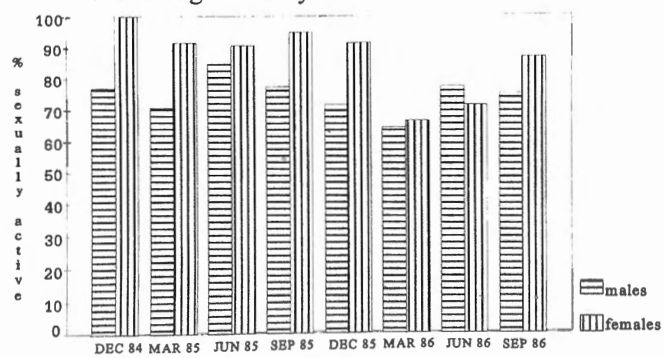

Fig. 3. – Percentage of male and female *P. jacksoni* sexually active.

Overall litter size is small in all species (*P. jacksoni*: 2.8 [n=113], *P. misonnei*: 2.3 [n=3], *P. mutoni*: 2.7 [n=3]) and pregnant females were found in each period for which observations were made.

DISCUSSION

Of the three *Praomys*-species that were found living in Masako forest, *P. jacksoni* was the most common and occured in all 4 habitats investigated. *P. misonnei* and *P. mutoni* were less abundant with a more restricted distribution. *P. mutoni* was found exclusively in riverine vegetation alongside rivers where *P. misonnei* was never caught. Further studies should indicate if this is due to interspecific competition or use of different resources (for instance food).

The biased sex ratio found for the three species may be due to the removal trapping technique used in which males, ranging over larger distances, have a higher probability of being trapped (FRYNTA & ZIZKOVA, 1994).

Reproductive characteristics seem to be very similar for the three species and reproduction is continuous throughout the year which is typical for equatorial tropical forest species (DELANY, 1964; DUPLANTIER, 1989).

P. jacksoni can be characterised as a K-selected species which is probably also the case for both other *Praomys*-species. The continuous reproductive activity, low litter sizes and high survival rates can be regarded as an adaptation to the constant forest conditions (climate, food availability etc.) (DELANY, 1972).

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THE BURDEN OF RODENT-BORNE DISEASES IN AFRICA SOUTH OF THE SAHARA

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Abstract. There are many vector-borne diseases in Africa which cause a heavy toll in human morbidity, mortality, economic loss and suffering. Plague remains endemic in several countries in Africa; 1,269 cases of plague were reported in Africa in 1994 and these represent 43.2% of the 2,935 human cases of plague from all the world and 50% of the mortality. The 6 countries which have reported human cases of the disease as recently as 1994 are Madagascar, Malawi, Mozambique, Tanzania, Zaire and Zimbabwe. Natural foci of the infection remain in others.

However, in addition to plague, other infections and human diseases with rodent reservoirs account for a great deal of morbidity and mortality in Africa though little actual data are available on the number of cases. The rodent-borne infections include the virus diseases Lassa fever, Crimean-Congo haemorrhagic fever, and, possibly, Rift Valley fever. Bacterial infections include brucellosis, leptospirosis, plague, rat-bite fever, tick-borne relapsing fever and tularemia. Rickettsial infections are common and include murine typhus, tick typhus, Q-fever. There are also several helminthic infections which may be passed from rodents to man and the most important of these is schistosomiasis. One must take into account the fact that serious rodent depredations on man's foodstuffs can also lead to malnutrition.

Key words: Africa, rodents, diseases, viruses, plague, leishmaniasis, infections.

INTRODUCTION

Rodent-borne infectious diseases are a serious burden on man's health causing a great deal of morbidity and mortality. Many such infections are found in Africa south of the Sahara and the following paper will review the diseases with rodent reservoirs or hosts known to be present in this part of of the continent and provide an overall view of what is known of the magnitude and public health importance of the infections.

It must be emphasized that many of the reports of infectious agents of this group include data from surveys in which rodents have been found serologically positive with antibodies for a particular infectious agent or in which a parasite has been identified in a rodent host; in a substantial number of cases, the epidemiology of the infectious agent and the role of the rodent as a reservoir is still unclear. As will be seen a great deal of research remains to be done to ascertain the importance of this group of diseases in both actual or potential rodent reservoirs as well as in man.

Table 1 lists the diseases with rodent reservoirs which have been reported from Africa with a listing of some of the rodent species or genera found positive.

TABLE 1

Rodent-borne diseases affecting man in Africa

Human disease	*Infectious agent*	*Rodent host(s) partial listing*	*vector (if any)*
Rodent-borne haemorrhagic fevers			
Crimean-Congo Haemorrhagic fever	Congo virus	*Mastomys* sp ?	*Hyalomma* ticks *Rhipicephalus* ticks *Boophilus* ticks
Lassa fever	Lassa virus	*Mastomys natalensis* (Smith, 1834)	none
Hemorrhagic fever with renal syndrome	Hantavirus	*Rattus* spp. *Mus musculus* L. 1758	none
Rift Valley Fever		*Arvicanthis niloticus* (Desmarest, 1822)	*Culex* mosquitoes
Rodent-borne viruses			
West Nile fever		*Acomys cahirinus* (Desmarest, 1819)	*Culex* mosquitoes
Quaranfil virus		*Acomys cahirinus* *Rattus* spp. *Mus musculus*	
Omo virus fever	Bunyavirus	*Mastomys erythroleucus* (Temminck, 1853)	*Onithodorous* spp.
Sandfly fevers	Saboya virus	*Tatera kempi* Wroughton, 1906	*Phlebotomus* sp.
		Mastomys sp.	*Sergenomyia* sp
Rodent-borne rickettsial diseases			
Spotted fever	*Rickettsia conorii*	*Mastomys* sp.	ticks
Murine typhus	*Rickettsia typhi*	*Rattus* spp.	*Xenopsylla cheopis* (Rothschild, 1903)
African tick bite fever	*Rickettsia africae*	?	ticks
Q-fever	*Coxiella burnetti*	*Acomys* sp. *Mastomys* sp.	direct contact
Rodent-borne bacterial diseases			
Brucellosis	*Brucella suis*	*Arvicanthis* sp. *Mastomys* sp.	direct contact
Rat bite fever	*Spirillum minus*	*Meriones* sp.	direct contact
Relapsing fevers	*Borrelia* spp.	*Rattus* spp. *Meriones* spp. *Arvicanthis* spp.	*Ornithodoros* ticks
Lyme disease	*Borrelia burgdorferi*	*Rattus* spp.	ticks

Leptospirosis	*Leptospira icterohaemorrhagie*	*Rattus* spp. *Arvicanthis* spp. *Cricetomys gambianus* Waterhouse, 1840 *Mus musculus*	direct contact
Plague	*Yersinia pestis*	*Mastomys natalensis* *Mastomys coucha* (Smith, 1834) *Rattus* spp. *Tatera* spp.	fleas
Salmonellosis	*Salmonella* spp.	*Rattus* spp	direct contact
Rodent-borne protozoal diseases			
Leishmaniasis	*Leishmania donovani*	Rattus spp *Tatera robusta* (Cretzschmar, 1830) *Arvicanthis niloticus* *Acomys cahirinus*	sandflies
	Leishmania tropica	*Acomys cahirinus* *Tatera* sp. *Arvicanthis niloticus*	
	Leishmania major	*Mastomys natalensis* *Mastomys erythroleucus* *Arvicanthis niloticus* *Aethomys kaiseri* (Noack, 1887) *Tatera kempi* *Tatera robusta* *Taterillus emini* (Thomas, 1892)	
Toxoplasmosis	*Toxoplasma gondii*	*Rattus* spp.	direct contact

That there are many rodent-borne diseases, emphasizes the close contact between man and the commensal and peridomestic rodent populations (GRATZ, 1988). However, despite the importance of this group of diseases and the fact that some have been recognized for a long period of time, their epidemiology and very distribution requires further study. This is particularly the case in Africa where, as will be seen, the group is of particular importance to public health and known to be the cause of much morbidity and mortality. Only the most important of this large group of diseases will be dealt with below.

RODENT-BORNE ZOONOSES

Viral infections

Antibodies to a large number of arboviruses (arthropod-borne viruses) have been detected in rodent populations of many different species and a smaller number of virus isolations have been made. As has been noted above, the actual importance of rodents for

many of these infections is unknown despite serological detection of a virus or even an isolation in a given species. Rodents are known to have a considerable importance as reservoirs of certain arboviruses such as Venezuelan Equine encephalitis in the Americas and Tick-borne encephalitis in Europe. For many of these infections, even the epidemiology relating to the disease in humans has yet to be elucidated.

There are about 500 arboviruses (arthropod-borne-viruses) known. Of this number around 100 cause clinical and subclinical disease in man. Some of these are of great public health importance such as yellow fever, dengue, dengue haemorrhagic fever, Japanese encephalitis, Rift Valley Fever, Crimean Congo Haemorrhagic fever and tick-borne encephalitis.

Haemorrhagic fevers

There is a long and growing list of haemorrhagic fevers being recognized from various regions in the world. There are 14 viruses that are considered to be haemorrhagic fevers. Mammals, especially rodents, are important natural hosts for many haemorrhagic fevers, (LEDUC, 1989).

Lassa fever

Lassa fever virus is known only from Africa. The disease which it causes was first recognized during an outbreak at a mission hospital in Jos, Nigeria in 1969. (FRAME *et al.*, 1970). In later investigations it was found that the reservoir of the virus is a rodent, *Mastomys natalensis* (Smith, 1834) whose distribution is widespread in Africa, (MONATH *et al.*, 1974, WULFF *et al.*, 1975) and no vector is known. The virus is spread directly from its rodent host through contamination of foodstuffs by rodent urine and excreta. The infection is now known to be present in many countries of West Africa and central Africa. There have been severe outbreaks of the disease in the last two years in Liberia and Sierra Leone, (WHO, 1996a). In Sierra Leone, Lassa fever accounts for 10% of all febrile illnesses admitted to hospitals and 1.7% of the general death rate (WHO, 1985). Outbreaks have also been reported from Burkina Faso, the Central African Republic, Côte d'Ivoire, Gambia, Ghana, Guinea, Mali and Senegal. Just how serious the disease can be was recognized in a recent study in hospitals and clinics in Imo State, Nigeria; a retrospective study showed that among 34 patients with Lassa fever whose number included 20 patients, 6 nurses, 2 surgeons and one physician and the son of one of the patients, there were 22 deaths, *i.e.* a case fatality rate of 65%. The attack rate in one of the hospitals studied was no less than 55% (FISHER-HOCH *et al.*, 1995)!

In a study of the rodent populations of Lassa fever patient's houses in Sierra Leone, 79% of the rodents caught were *M. natalensis* (KEENLYSIDE *et al.*, 1983). Of this number no less than 39% were viremic. In an effort to control transmission, rodent were trapped in half the case houses but this failed to reduce the seroconversion rate. MCCORMICK *et al.* (1987) also studied the prevalence of Lassa virus in *M. natalensis* in Sierra Leone; this species constituted 50 to 60% of the rodent species captured in houses in the villages but only 10 to 20% of those trapped in the surrounding agricultural areas. Virus prevalence

ranged from 0% to a high of 80%. They calculated that the ratio of fatalities to infection in humans infected with Lassa was about 1 to 2% but the high incidence of the disease makes it a major public health problem in West Africa.

During 1996, a total of 470 cases with 110 deaths (23.4% CFR) were reported from Sierra Leone. Four of these cases were reported from Freetown. In the first four months of 1997, there were a total of 353 cases of Lassa fever with 43 deaths though the civil unrest at the time of writing prevents full reporting (WHO, 1997b). The increased number of cases has been ascribed to crowded conditions, poor sanitation and an increase in the rodent populations (WHO 1997a).

Strains of a closely related virus have been isolated in Mozambique (WULFF *et al.,* 1977) and Zimbabwe (JOHNSON *et al.,* 1981). The virus in both countries has been given the name Mozambique virus. In Zimbabwe it was isolated from both *M. natalensis* and *Aethomys chrysophilus* (de Winton, 1897). It seems quite likely that the infection, or closely related viruses are more widely spread than presently reported but further serological studies must be carried out.

Mobala Virus

This relatively new arenavirus has been isolated from *Mastomys natalensis* and *Praomys* sp. in the Central African Republic; its public health importance and distribution outside of the CAR are, as yet, unknown, (GONZALEZ *et al.,* 1984).

Hantavirus disease-Haemorrhagic fever with renal syndrome

This group of infections caused by a group of hantaviruses is known to be present in many countries in the world and appears to be spreading or being recognized from countries in which they have not been know previously. The infections are known under several names such as Korean Haemorrhagic fever, Tula virus and others but in most cases these are the same virus with different levels of virulence. In China and eastern Russia, the infection is responsible for large numbers of cases and many deaths every year. Recently described new members of the group in the USA and Europe have caused a high level of mortality and great concern, (GLIGIC *et al.,*1992, NILKLASSON *et al.,* 1995, ROLLIN *et al.,* 1995, WARNER, 1996). These newly recognized hantaviruses have been given different names among them Bayou virus, Sin Nombre virus. In Africa, the infection is widespread in human and rodent populations and has been reported in Benin, Burkina Faso, Cameroon, Central African Republic, Chad, Equatorial Guinea, Gabon, Mauritania, Madagascar, Nigeria, Senegal, Uganda, (FISHER-HOCH & MCCORMICK 1985) and Tanzania, in the latter from the island of Zanzibar (NUTI & LEE, 1991). It appears very probable that the virus will be found almost wherever it is looked for but very little information is available on its prevalence, clinical picture or public health importance in man in Africa.

Rift Valley Fever Virus

Rift Valley fever is known to extend from South Africa northward through Kenya and Sudan and to west Africa. Large, epidemic, outbreaks of the disease have occurred in

Egypt in 1977-1978 and 1993 with a large number of deaths and abortions among herds and some 600 human deaths in the first outbreak. The vector of the disease is a mosquito and the identity of the reservoirs is still uncertain. It has been suggested that rodents are one of the reservoirs of RVF but this requires further verifications, (SCOTT & HEISCH, 1959). KEOGH and PRICE (1981) in their review of the multimammate mice expressed doubt as to whether this group could be the reservoir for RVF.

Rabies or rabies like infections

It has been commonly accepted that rodents do not serve as reservoirs of rabies; there has, however, been a report from South Africa of the presence of a rabies like virus, Mokola virus, in rodents (SWANEPOEL *et al.*, 1993). The significance of this is not yet entirely clear.

Rickettsial diseases

Spotted fevers or «African tick typhus»

In Africa south of the Sahara, the seroprevalence of the spotted fever group of rickettsial infections is higher than anywhere else in the world, (DUPONT *et al.*, 1995). This infection caused by *Rickettsia conorii* , is widely spread throughout Africa. The infection is relatively mild with a low mortality of less than 3% even without treatment. The vectors are ticks of several different genera and strains of *R. conorii* have been recovered in South Africa from *Otomys irroratus* (Brants, 1827) and *Rattus rattus* (L., 1758). A large number of species have been found positive in Kenya including *Arvicanthis niloticus, Rattus rattus, Mastomys natalensis, Aethomys kaiseri, Lophuromys flavopunctatus* Thomas, 1888 and *Lemniscomys striatus* (L., 1758). Rates of human infection may be quite high; in Sierra Leone and the Côte d'Ivoire, 7% of the population was found to be seropositive to rickettsial diseases in some areas, mainly for spotted fever (REDUS *et al.*, 1986) while in Central Africa and Zimbabwe the rate may be as high as 45% (BROUQUI *et al.*, 1992).

Murine typhus

Murine typhus caused by *R. typhi*, is probably present throughout most of coastal Africa or inland cities where the main reservoirs *Rattus rattus* or *Rattus norvegicus* (Berkenhout, 1769) and flea vectors of the genus *Xenopsylla* are present. Little information is available on the incidence of infection with murine typhus. DUPONT (1995) found that antibodies to *R. typhi* ranged from 1% to 20% in the populations studied; as elsewhere, where commensal *Rattus* species are common, murine typhus infections will probably be frequent as well though most of them will go undiagnosed. The disease is relatively mild but an infection usually necessitates a long period of convalescence.

Bacterial diseases

Several of the bacterial diseases with rodent reservoirs in Africa are responsible for a very considerable morbidity and significant mortality. The relapsing fevers, plague and

leptospirosis are important from a public health viewpoint in many foci throughout Africa and the continent has not escaped the spread of Lyme disease.

The various species of *Borrelia*, the agents of relapsing fever, all have tick vectors and of this group of diseases, only the „crocidurae" group have rodent reservoirs. The infection is widely distributed from Senegal to Kenya. In West Africa the infection is classically limited to the Sahel and Saharan region and the reservoirs are peridomestic *Cricetomys gambianus, Rattus rattus* and *Arvicanthis niloticus* among others. TRAPE *et al.* (1991) suggest that the disease is not only very widely spread in Senegal but throughout all of Africa.

Lyme disease

Lyme disease was first recognized in the eastern USA in 1975 though it is now realized that the infection was actually known by its clinical characteristics in Europe from early in the century. The vectors of the infectious agent, the spirochete *Borrelia burgdorferi*, are ticks of the genus *Ixodes*. The disease resulting from the infection can be quite severe and is difficult to treat. The disease has become the most important vector-borne disease in North America and has spread widely in Europe and Asia. Apparently the infection was first recognized in South Africa in 1989 and cases have now been reported from both east and west Africa. Though rodents are known to be the most important reservoir elsewhere, little information is available on the reservoir species in Africa. It would appear, however, that as elsewhere, the infection is spreading and research should be undertaken to determine the rodent hosts. It is reported that *Rattus norvegicus* and *Rattus rattus* are the reservoirs of Lyme disease on Madeira Island (MATUSCHKA *et al.,* 1994).

Leptospirosis

Leptospirosis is the most widespread zoonoses in the world. The disease in man, though usually mild, can be quite severe with significant mortality in older people. While many different animals can serve as host to the leptospires, rodents are frequently the source of *Leptospira icterohaemorrhagiae* passing the infection to man through their urine; the infective agent in rodent reservoirs has been reported from many countries in Africa including Benin, Cote d'Ivoire, Kenya, Madagascar; Mali, Reunion, Senegal, Seychelles, Tanzania, Uganda, and Zimbabwe and will probably be found anywhere where there are populations of commensal or peridomestic rodents in close contact with man. The infection in the Seychelles was studied over a two-year period when 80 cases were diagnosed at Victoria Hospital with a 16% mortality; it was considered that the infection was primarily *Rattus* species borne (PINN, 1992). The rodent hosts found positive with Leptospires in Africa include *Arvicanthis niloticus, Cricetomys gambianus, Mastomys* sp., *Mus musculus* and, particularly, *Rattus norvegicus* and *R. rattus* (FIEDLER, 1988). The first human case of leptospirosis has only been recently described from Gabon and confirmed serologically. The rodent fauna of the country is rich, houses are heavily infested and pigs and dogs circulate freely in the villages (PERRET *et al.,* 1994). There seems little doubt that further surveys will find the infection widespread. Studies in Benin (KOUNDE, 1996) showed a high percentage (51.7%) of the rodents examined, mainly *Rattus norvegicus*, positive for leptospires. FERESU & DALU (1996) recently isolated 49 strains of *Leptospira*

in the city of Harare, Zimbabwe, 43 from *R. rattus*, 2 from *Mastomys* sp. and 4 from *Mus musculus*.

Plague

The causative agent of plague is *Yersinia pestis*. The infection is passed from one rodent to another by fleas which have fed on an infected rodent. The infection is maintained in sylvatic rodent populations in natural foci of the disease over long periods of time. If fleas infected from rodents in these foci feed on man, the disease can then be passed on to humans. If untreated the disease can cause a high mortality, particularly if the pneumonic form of the infection develops. If sylvatic rodent fleas feed on peridomestic or commensal rodents, an outbreak of plague can occur in human settlements. Seven countries in Africa have reported plague in 1993 and 1994 as shown in Table 2.

In recent years outbreaks have also been reported in Botswana and Kenya, and probably remains endemic in other countries as well. While the number of cases of human plague is not great as compared with that of other vector and rodent-borne diseases in Africa, the disease is doubtlessly considerably undereported in Africa and the potential for serious outbreaks with a high case fatality rate due to delayed diagnosis and reporting is significant. The principal rodent reservoir in Africa is the multimmate rat, *Mastomys natalensis*. Many different species have been found infected with plague but the principal reservoir species are *M. natalensis* and *Tatera brantsii* (Smith, 1836) in southern Africa. HALLETT and ISAACSON (1975) noted that a rodent die-off preceded a human outbreak if plague in South Africa and that *Otomys unisulcatus* Cuvier (1829) was an important rodent in the plague cycle. SHEPHERD *et al.* (1983) also noted that a rodent epizootic appeared to precede a human outbreak in eastern Cape province in 1982. The rodents involved were *Rhabdomys pumilio* (Sparrman, 1784) and *Otomys irroratus*. In Madagascar, the only reservoir of plague is *R. rattus*, (BRYGOO, 1966); the changing ecology of the country with the destruction of forest habitats, favors the spread of this species The disease is endemic in about 15% of the country and there is evidence that the strains of *Y. pestis* are becoming more virulent, (MICHEL *et al.*, 1989). Both bubonic and pneumonic forms are now appearing and many of the pneumonic cases die. Between 1989 and 1992, 312 cases of plague were serologically confirmed in Madagascar and another 335 were considered as probable. Of these cases, 93% came from the „central triangle" with cases occurring throughout the year but mainly during the rainy season from November to March, (BLANCHY *et al.*, 1993). Two outbreaks have occurred in Mahajanga harbour in 1991 and 1995-1996 where plague had earlier disappeared in 1928. After the human epidemic, the shrew *Suncus murinus* (L., 1766) represented about 90% of captures and one shrew was infected by *Y. pestis* and the role of this species requires further investigation (DUPLANTIER *et al.*, 1996).

The cases which occurred in Mozambique are the first to be reported in that country in 15 years and they occurred in Tete Province which is known to be plague-endemic. Investigations on the plague reservoirs in Zimbabwe during an outbreak in 1983 showed that *Tatera leucogaster* (Peters, 1852) and *Mastomys coucha* were very susceptible to *Y. pestis*, usually dying quickly after infection which makes it unlikely that they can act as

the reservoirs. *Aethomys chrysophilus* and *Mastomys natalensis* are relatively resistant to the infection and are much more probable sylvatic reservoirs. Both species of *Mastomys* are semi-domestic in their habits and may act as a link between man in villages and the true sylvatic foci (WHO, 1983).

TABLE 2

Human cases of plague in Africa in 1993-1994. Deaths indicated between parentheses (WHO, 1996b)

Country	*1993*	*1994*
Madagascar	127(23)	126 (15)
Malawi	9	-
Mozambique	-	216 (3)
Tanzania	?	444 (50)
Uganda	167 (18)	-
Zaire	636 (89)	82 (10)
Zimbabwe	-	392 (28)

As can be seen in Table 2, the largest number of cases in Africa have been reported from Tanzania. KILONZO (1992) in a survey of 6 regions of the country, found that of 5,638 small animals captured, 2.4% contained agglutinating antibodies for the infection. Antibody positive rodents were found in Lushoto, Mbulu, Chunya and Monduli districts and in Tanga seaport. The disease may be spreading in Tanzania as KILONZO & MHINA (1982) described a plague epidemic of 49 cases and 11 deaths in the Tanga region in 1980 where the disease had never been recorded before. Further investigations on endemic areas in Tanzania and improved surveillance, both laboratory and clinical are a priority.

Much research remains to be carried out on the epidemiology of plague in Africa particularly on the nature of the sylvatic foci that enable plague infections to persist over long periods of time. The continuing presence of such natural foci of plague imply a constant threat of outbreaks of the disease among human populations. SHEPHERD & LEMAN (1983) found three species of rodents antibody positive in South Africa, *Desmodillus auricularis* (Smith, 1834), *Tatera brantsii* and *Rhabdomys pumilio*, and positive rodents were found in 1972, 1974, 1975 and 1979 showing that the infection continues to circulate over long periods of time even in the absence of human cases.

Protozoal diseases

Leishmaniasis

The causative agents of the various forms of leishmaniasis are protozoa of the genus *Leishmania*. The vectors are all sandflies of the genera *Phlebotomus* and *Sergentomyia*. Most of the leishmaniasis are zoonoses and among the most important of the reservoir vertebrate hosts are rodents. There are two main clinical forms of the disease which may be caused by one of several species. Visceral forms of the infection frequently cause of death

if untreated. Endemic visceral leishmaniasis, or kala azar, in East Africa is caused by *L. donovani*. In Kenya parasites of *L. major* causing dermal or cutaneous leishmaniasis have been isolated from *Tatera robusta, Arvicanthis niloticus, Mastomys natalensis, Taterillus emini* and *Aethomys kaiseri* (GITHURE *et al.,* 1986).

Visceral leishmaniasis or Kala azar is a serious public health problem in Sudan and is the cause of much morbidity and mortality. The infection is spreading to many areas where it was not previously known to be present. Surveys in the Upper Nile Province of southern Sudan have found *Arvicanthis niloticus* and *Acomys cahirinus* positive for *L. donovani*, (EL-HASSAN *et al.,* 1993). PEREA *et al.,* (1991) carried out a study in the same general area and found a prevalence of *L. donovani* infection of 18.2% and believe that the disease has already killed thousands of persons and is spreading. ASHFORD & THOMSON (1991) believe that the outbreak of visceral leishmaniasis in the western Upper Nile province has killed at least 30,000 people and largely depopulated an area some 50 km in diameter. The great ecological changes in the area, in part as a result of the civil war, may be an important factor in the increase and spread of the infection.

There has also been a great increase in cutaneous leishmaniasis due to *L. major* in Sudan resulting in a major epidemic along the Nile River north of Khartoum in 1985. KADARO *et al.* (1993) sampled the human population and found that 4% had active lesions of cutaneous leishmaniasis, 47% has healed lesions and another 43% had positive reactions to a sensitization test though they showed no lesions. All in all, they found that 91% of the population has a positive reaction. They believe that one of the factors in this very high rate is the increased population density of *Arvicanthis niloticus*.

In west Africa, *L. major* has also been isolated from the livers and spleens of *Mastomys natalensis* and *Tatera kempi* in northern Nigeria (IKEH *et al.,* 1995). As further studies are done, it seems likely that growing recognition will be given to the public health importance of leishmaniasis and hence of the rodent reservoirs of the infection in Africa.

Rodent-borne worm infections

Schistosomiasis

Although schistosomiasis is an important public health problem throughout Africa, the role of rodents as reservoirs of the infection has only been briefly investigated. KAWASHIMA *et al.* (1978) found that in the Taveta area of Kenya *Pelomys* sp. was infected by *Schistosoma mansoni* (Sambon, 1907) and suggested that it might play a role as a reservoir in this area.

In recent studies in a relatively new focus of *S. mansoni* in Senegal, 5% of *A. niloticus* and *Mastomys huberti* (Wroughton, 1908) trapped in ricefields and orchards were positive. While the prevalences and worm loads were low, the Richard-Toll focus is only seven years old and may increase in the future, (DUPLANTIER & SENE, 1996).

Other worm parasites of man have been found in rodents in Africa such as *Capillaria hepatica* in 19 out of 308 (6.2%) *Rattus rattus* and 1 out of 312 (0.5%) *Myomys albipes* (Rüppell, 1842) in Ethiopia (FARHANG-AZAD & SCHLITTER, 1978) and in 48% of *M. natalensis* in South Africa. Trichinosis parasites have also been found in several rodent species

in Africa but the extent to which this infection and other worm parasites found in rodents are important public health problems in man is unknown in Africa.

CONCLUSIONS

Despite the comparative lack of accurate data on the human incidence of most of the rodent-borne diseases in Africa, enough information is available to show that as a group they constitute a serious burden on the human population in those areas in which they are endemic. To the burden of infectious diseases one must add the effect on the nutrition of man which results from rodent depredations on foodstuffs; while relatively little information is available on just how serious these are in economic terms, the many examples presented by FIEDLER (1988) give good reason to believe that the losses in cash and food crops adversely affect the availability of food on a continent where adequate nutrition is already a problem.

It is essential to improve the surveillance of the rodent-borne diseases in Africa; only with more accurate information on their magnitude can one judge the resources that must be devoted to control of the rodent reservoirs. Such information, were it available, would also provide a guide to the areas where greater emphasis must be placed on the control of the reservoir species. Increased surveillance on the incidence of this group of diseases in man must be linked with more detailed studies to determine which rodent species are the most important reservoirs. Studies on the bionomics of the most important rodents will also enable more selective, effective and economic control measures to be undertaken.

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WHAT IT TAKES TO BE A RESERVOIR HOST

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Abstract. The majority of parasitic infections of man are of zoonotic origin. In a conceptual framework for the incrimination of mammalian reservoir hosts, these are defined as being essential to the maintenance of parasite suprapopulations. A series of guidelines are given for the accumulation of the relevant information. It is proposed that mammal ecologists can contribute significantly to the understanding of medically important zoonoses.

Key words: zoonoses, reservoirs, epidemiology.

INTRODUCTION

Zoonoses, diseases transmitted between man and other animals are of great public health importance, so have been the subjects of much investigation. Many such infections are highly pathogenic and, while most occur only sporadically, some are responsible for important epidemics. Zoonoses with wild animal reservoir hosts often occur focally, obeying Pavlovsky's rules of «natural nidality» (PAVLQVSKY, undated). Controlling the transmission of such zoonoses may depend on the description of the natural reservoir, and on a deep understanding of the ecology of the reservoir host(s). This article is intended to contribute to a conceptual framework for the study of eukaryote zoonoses by describing general features and providing definitions and guidelines for the incrimination of reservoir hosts. Microbial zoonoses are not included; while these obey similar rules, the study of prokaryotes requires a set of different methods.

A CONCEPTUAL FRAMEWORK

Zoonoses

At the latest count (Unpublished result based largely on BEAVER *et al.*, 1984 and COOMBS & CROMPTON, 1991) some 374 species of eukaryote have been recorded as natural parasites of *Homo sapiens* L., 1758. Among these, no fewer than 299 are thought to be purely zoonotic. That is, their suprapopulations are never dependent on *H. sapiens* for their long term survival; human infection is derived directly or indirectly from another species of vertebrate. A further 31 forms are partially zoonotic, being maintained by *H. sapiens* as well as other hosts. Only 44 species are regarded as being entirely dependent on man. This last number is likely to fall with increasing information, while the other two are likely to increase.

Most zoonoses are derived from natural mammalian hosts. Fig. 1 illustrates the mammalian orders and the numbers of zoonotic parasitic infections maintained in each one. Relative to the number of species in the order, the Carnivora and ungulates are the most important sources of human infection, but the Rodentia are also very important. For a considerable number of presumably zoonotic infections, the reservoir host is unknown

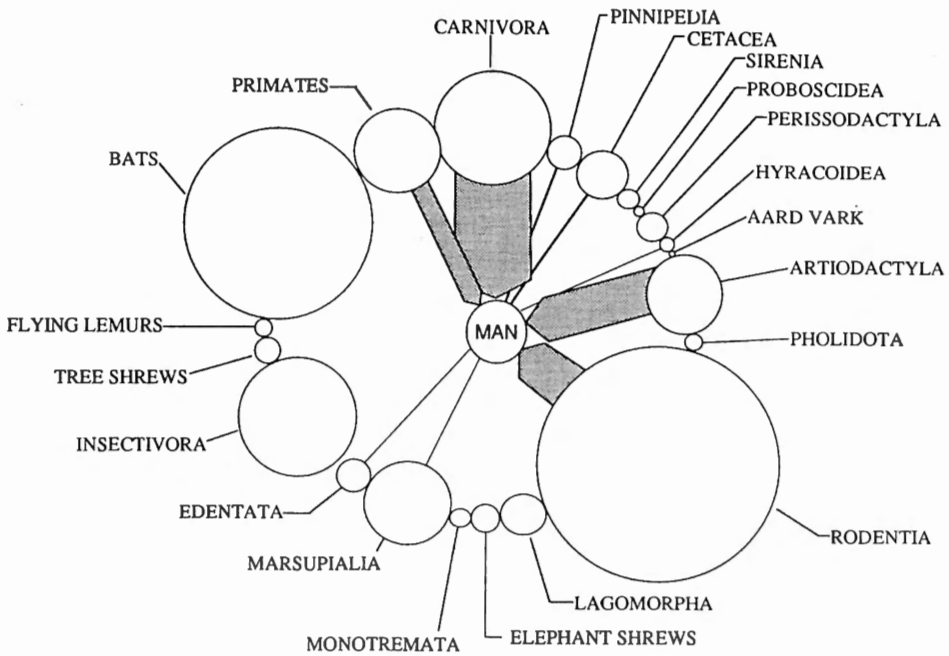

Fig. 1. – Mammalian reservoir hosts for zoonotic endoparasites. The area of each circle is proportional to the number of species in the order; the width of each arrow is proportional to the number of zoonotic endoparasitic infections harboured by the species of the order.

The concept of zoonosis is strictly anthropocentric so, in itself, has no general scientific relevance. However, the economic importance of zoonoses has led to much research on them and they may be used as paradigms illustrating many general parasitological principles. Thus, a reservoir host of a zoonotic infection is a maintenance host of a parasite which also infects another host species, and the structure of reservoir systems can readily be generalised to describe parasite - host systems in general.

Reservoir Systems

A reservoir of infection is best defined as an ecological system in which the infectious agent survives indefinitely. Where a vertebrate host or group of hosts is essential to such a system, these are termed the reservoir host(s).

For relatively specialised parasites such as *Leishmania aethiopica* the reservoir system may comprise one or a few reservoir hosts (the hyraxes *Procavia* spp. and *Heterohyrax* brucei [Gray, 1868]) For generalist parasites such as *Toxoplasma gondii* or *Trichinella spiralis* (Owen, 1835), the system may include numerous reservoir hosts in any one place, and these may vary geographically. It is important to distinguish between hosts which are essential to the system and those which are merely incidental. That is, those which form part of an ecological source and those which are merely ecological sinks.

To be an essential component of a reservoir system, the vertebrate host must at least be susceptible to infection and the agent must reach its transmission stage. The other features of reservoir hosts are less easy to define or measure, and depend on long term quantitative interpretation. At all stages the whole reservoir system must be considered and it is only when the system has been described at least semi-quantitatively that a final assessment can be made. The question is particularly intractable with generalist parasites for which any one host species may be insufficient to ensure long term persistence of a suprapopulation.

In order to satisfy the requirements of a reservoir host, an infected individual must, on average, be responsible for the subsequent infection of at least one other individual. As shown schematically in Fig. 2 it is hypothetically possible for the reservoir system to include more than one reservoir host species. In the example given, based on *T. gondii*, the reservoir system includes the cat as definitive host and various rodents and birds as intermediate hosts. Suppose the average infected cat infects one each of *Apodemus, Arvicola, Mus, Rattus* and *Passer*; then each of these in turn infects 0.25 cats, so a total of 1.25 cats are subsequently infected and the cycle can continue. However, no single intermediate host species is either essential or sufficient to maintain the system. In this example any four of the five intermediate hosts are required: all must be termed reservoir hosts. Extending this hypothetical example, each infected individual of *Rattus* spec. has a small

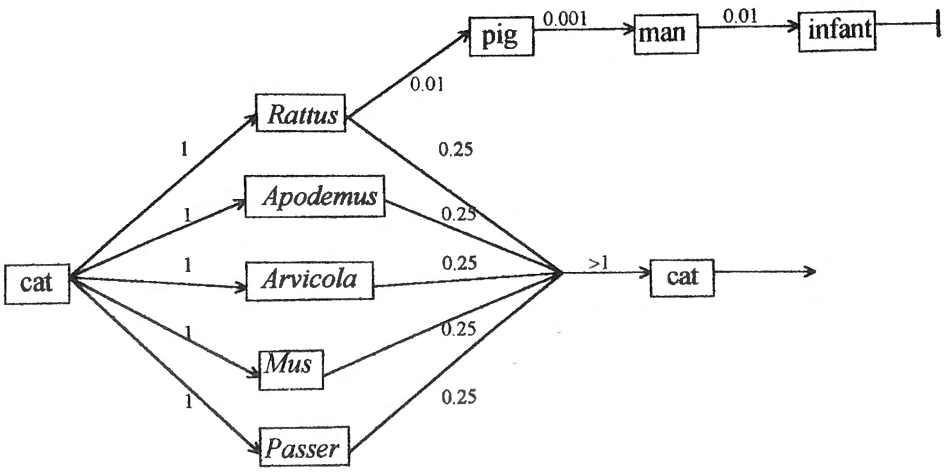

Fig. 2. – Hypothetical scheme for a complex reservoir system in which more than one reservoir host is required. Based on the life cycle of *Toxoplasma gondii*, but figures are for illustration only. See text for explanation.

probability of infecting a pig which, in turn, has a small possibility of infecting a human. The pig is most unlikely to infect a cat or, in any other way, to contribute to the maintenance of the system, so is not a reservoir host. From the anthropocentric point of view, the pig is an important source of human infection. Such a host, which causes humans to be exposed to infection, but plays no part in the maintenance of the reservoir system is termed a liaison host (GARNHAM , 1971).

Incrimination of Reservoir Hosts

The ideal goal in describing a zoonotic disease would be to enter all the numbers on a diagram resembling Fig. 2. However, there is probably no zoonosis whose ecology is sufficiently well known to measure all the parameters required to formally construct such a comprehensive mathematical model. Fortunately however, quantitative information on parts of the system is frequently sufficient that 'intuitive' interpretation may be quite convincing. Further, at this level of complexity (where accuracy risks becoming divorced from precision) it is doubtful that any biomathematical model could be more reliable than informed 'intuition'. It must be emphasised that the fact that comprehensive mathematical models are of limited value in no way denies the essential value of quantitative data, or of models of small parts of systems.

In practice, the measurement of various parameters is possible which are sufficient to incriminate most reservoir hosts with reasonable certainty. This can be illustrated by the various zoonotic species of *Leishmania*, which show a wide variety of patterns within a homogeneous group of parasites. The roles of mammals in the maintenance of Leishmaniasis systems has been reviewed by ASHFORD (1996).

Selection of field study site

This usually depends on the location of cases of human infection, preferably foci in which many cases occur. Travel histories are vital; it is important to visit homes and carefully interview subjects. Age and occupation risk factors may be strong indicators of specific transmission sites; infection in infants frequently indicates peridomestic transmission, so is especially valuable. This information can usually be gathered from existing records and informal open questionnaire, without resort to formal epidemiological investigation. Epidemics in which humans are temporary sources of human infection may be very misleading. In southern Sudan epidemics of *L. donovani* occur over a wide area but these seem to be anthroponotic; there appear to be residual foci of zoonotic infection in sparsely inhabited areas, whose structure remains to be described, which cannot readily be located during epidemics. Strong parallels exist in this respect with plague.

Collection and incrimination of candidate reservoir hosts

The importance of accurate identification of the vertebrate host cannot be overemphasised. Candidates may be chosen according to *prima facie* evidence. A maintenance host for one *Leishmania* species is likely to be a good host for others; it is likely to constitute a large proportion of the mammalian biomass, at least in restricted areas, so is either abundant or gregarious. Alternatively there may be a specific association between a sandfly

vector and vertebrate host which increases the chances of transmission. This has been found for sloths *Choloepus* and *Bradypus* spp, which maintain *L. panamensis* and *L. guyanensis*. Experimental infection may be very misleading; many workers have found great difficulty in infecting natural hosts with cultured *Leishmania* parasites. If the season and age group of maximum prevalence can be predicted, the number of animals needed to be examined in order to exclude a candidate is unlikely to exceed 100.

Detection of parasite

Here again taxonomy and identification present real difficulties. Workers in central Asia misjudged the risk of zoonotic *L. major* infection for many years before it was discovered that they were dealing with two species of parasite, *L. major*, which infects humans and *L. turanica* which does not. The results of a massive effort by the American Navy in southern Sudan in the 1960s are uncertain today owing to doubt about the identity of the parasites they isolated from *Arvicanthis niloticus* (Desmarest, 1822). Biochemical methods of classification and identification have contributed greatly in recent years. Wherever possible parasites must be isolated in culture for detailed identification. It is important to establish that the parasite reaches a stage in which it can be transmitted.

Estimation of parasite population parameters

The most important parameters are prevalence, incidence and duration of infection. Incidence, which is the most expensive parameter to measure, can be estimated as prevalence / duration, but care must be taken to allow for seasonal effects. The most valuable practical measure for most host - parasite combinations is the relation between host age and prevalence of active or past infection. The use of eye lens weight as a measure of the age of rodents has proven to be of immense value in our own (unpublished) study of *L. major* in *Psammomys obesus* Crezschmar, 1828 in North Africa.

Estimation of host population parameters

The main population factors favouring a reservoir role are high density, and longevity sufficient to provide a habitat for the parasite during any non-transmission season. Many of the normal topics of mammal ecology studies, such as nutrition, are of limited interest in medical mammalogy. Some parameters can only be measured with longitudinal study of a population by removal. This can conveniently be combined with parasitological study as described above. Mobility and longevity can, however, best be measured by mark-capture-recapture methods without removing the animals, which is incompatible with parasitology. Indirect methods such as serology may overcome this problem, but are only valid when the structure of a focus has been qualitatively described.

Effect of parasite on host

The old idea that a 'well adapted' parasite does not harm its host is clearly not a valid generalisation: many parasites depend on the death of their host for transmission, and cer-

tainly accelerate this death. Nevertheless, it is generally difficult to demonstrate serious effects of common parasites on individual mammalian hosts. Any effect on natural host populations is even more difficult to demonstrate. *Leishmania* species in their natural hosts may infect the skin or viscera; infections last for the life of the host, but are not known to cause significant pathology, nor to reduce ecological fitness. Cutaneous infections are frequently undetectable visually. It is only the domestic dog, which must be a secondary reservoir host of *L. infantum*, and man, as a presumed secondary maintenance host of *L. donovani*, which suffer serious disease.

The measurement of effects of parasites on natural host populations requires extended longitudinal study, and such effects have rarely been demonstrated. Reservoir hosts are, by definition, maintenance hosts so infection is likely to be either too infrequent or too benign to have any regulating effect on populations. An acutely pathogenic parasite is likely to be in an unusual host species.

CONCLUSION

The identification of reservoir hosts is an essential component of programmes for the control of zoonoses. Only with a thorough understanding of the basic ecology of the host will efficient control be possible and, with parasitic zoonoses, there have been very few comprehensive ecological studies aimed at the reduction of transmission to man. Mammalogists have tended to measure parameters relevant to basic ecology or crop protection, without reference to zoonotic parasites. Parasitologists have tended to examine large numbers of hosts haphazardly, without reference to their population structure. Carefully designed studies of the interaction between host and parasite populations are called for. These are expensive and, meanwhile, mammalogists should be more aware of parasitology, and parasitologists should be more meticulous in the gathering of data concerning the hosts they are studying. Microbial zoonoses such as rabies and plague are much more thoroughly understood in this respect, and interaction between the relevant experts would be highly productive, initially to develop a consistent terminology for the subject of zoonoses as a whole.

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THE LABORATORY DIAGNOSIS OF PLAGUE

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Abstract. Several diagnostic methods for *Yersinia pestis* infections are available including culture, serological, molecular and chromatographic assays. These techniques may be applied to human clinical specimens as well as rodents and other animals sampled in the course of plague surveillance. All the methods have their merits depending on the time available and the information required. Time is of the essence in the diagnosis of plague. Rapid diagnostic techniques capable of detecting *Y. pestis* directly in clinical samples, infected animal tissue and fleas will facilitate speedy diagnosis. The culture methods are reliable but relatively slow and insensitive. The serological ELISA tests are sensitive but in the case of the antibody assay, rely on a detectectable humoral immune response. Antigen can be detected at an earlier stage in the infection. DNA probes lack sensitivity, as 10^5-10^6 organisms are needed for reliable detection. The PCR is sensitive and can detect as few as 10 *Y. pestis* organisms, but cannot distinguish between live and dead bacteria. A recently-developed chromatographic assay is very specific and sensitive and takes only 10 minutes to obtain a result, but thorough field testing is awaited.

Key words: plague, *Yersinia pestis*, diagnosis, culture, serology, molecular biology, polymerase chain reaction.

INTRODUCTION

Plague is an acute systemic infection caued by *Yersinia pestis*. The infection, known since antiquity, has caused three authenticated pandemics. The third pandemic, after a slow advance from its origin on the Central Asian Plateau, reached the Chinese coast in the late 19th century. Throughout history a connection between rat mortality and the appearance of human plague has been noted (WU LIEN-THE *et al.*, 1936). According to excerpts from his diary quoted by LAGRANGE (1926), Alexandre Yersin, after whom the organism was named, detected plague in Hong Kong rats in 1894 and demonstrated the causative organism. Plague spread rapidly around the world from China and new wild rodent plague reservoirs were established in many parts of the world, including South America, the western United States and southern Africa. Plague diagnosis, surveillance and control have subsequently been researched in South Africa for more than 80 years. Laboratory diagnosis of plague is required for both human cases and for plague surveillance. In this brief review we emphasize the methods used in our own laboratory, and mention potentially useful new techniques.

METHODS

Culture

Smears of clinical material can be prepared under almost any circumstances and are very important in the identification of the organism. The plague bacilli can be demonstrated, often in large numbers, in these smears. *Y. pestis* is a Gram-negative cocco-bacillus that ranges from 0.5-0.8 μm in diameter and 1-2 μm in length and demonstrates typical bipolar staining with Grams or Waysons stains. Blood, bubo aspirates and sputum may be cultured for the presence of *Y. pestis*. It is important that clinical specimens are taken before antimicrobial therapy has been started. These samples are plated onto blood agar and incubated at 28° C for up to seven days. *Y. pestis* is a fairly slow-growing organism but will grow quite satisfactorily on a variety of ordinary media. The optimal growth temperature is 28° C, but growth can be obtained at temperatures ranging between 2° C and 45° C. In culture, the organism shows pleomorphism depending on the medium and temperatures used. The colonies of *Y. pestis* are opaque, smooth and round, although in some instances, irregular edges have been noted. When grown in liquid medium *Y. pestis* typically forms a deposit at the bottom of the tube and the supernatant remains relatively clear. *Y. pestis* is catalase-positive but oxidase-negative and is non-haemolytic. The organism is identified in biochemical tests, *e.g.* these tests demonstrate acid but not gas from glucose, mannitol and salicin; there is no acid reaction in sucrose, rhamnose and melibiose and the Voges-Proskauer test is negative. For confirmation *Y. pestis* is lysed by specific bacteriophage at 20° C (BAHMANYAR & CAVANAUGH, 1976). Various virulence factors can be demonstrated by using special media but this is not required for routine identification. Overgrowth of *Y. pestis* by other bacteria may be circumvented by animal inoculation. Ordinary white mice or guinea pigs can be used but WILLIAMS *et al.* (1982a) demonstrated the advantage of using laboratory-reared African multimammate mice (*Mastomys coucha* (Smith, 1834), 36 chromosome species) in the diagnosis of plague. Several atypical phenotypes of *Y. pestis* could be isolated from *M. coucha* after experimental inoculation but some could not be recovered from the other rodent species tested. If the material being cultured is likely to be contaminated with other organisms, it is diluted 1:10 with normal saline and inoculated intraperitoneally into two female *M.coucha*, following intraperitoneal injection of 100 g of Fe_2SO_4. At 48 hours post-inoculation, one of the rats is killed by CO_2 inhalation. Isolation of *Y. pestis* by streaking impressions of freshly cut portions of spleen and liver onto blood agar plates is attempted. The second female rat is killed 72 hours post-inoculation, and isolation of *Y. pestis* is likewise attempted. Triturated reticuloendothelial organs, especially bone marrow, of dead rodents collected in the course of surveillance activities, are usually best inoculated into laboratory animals as a first step in culturing *Y. pestis*. A useful method of transporting dead rodents to the laboratory for attempted plague bacillus isolation, particularly if delays are anticipated, is to first dust them with insecticide and then pack them in salt in screw-cap jars as described by BALTAZARD *et al.* (1956). Great care must be taken to avoid laboratory-acquired infections when culturing *Y. pestis* and all such work should ideally be done in a class 2 biohazard hood. Likewise, fleas from susceptible hosts pose a plague transmission risk for field and laboratory staff and adequate precautions must be taken when working with them.

Serological tests

The first specific plague haemagglutination test was developed by CHEN & MEYER (1954). Passive haemagglutination (PHA) relies on tanned sheep red cells, sensitised with F1 antigen. WILLIAMS *et al.* (1982b) initiated comparative studies using the PHA test and the F1 enzyme-linked immunosorbent assay (ELISA) for the confirmation of clinically suspected human plague; the latter was shown to be much more specific and sensitive. Likewise, SHEPHERD *et al.* (1984) demonstrated that by using an F1 ELISA, the number of non-specific reactors in dog sera were cut by almost two-thirds compared to PHA. PHA does however have the benefit of not requiring genus-specific anti-immunoglobulin, making it simple and cheap, especially for field surveillance where the same test can be applied to many different species of animals. ELISA has the major advantage that specific IgG and IgM titres can be determined, from which conclusions can be drawn regarding the period of time elapsed since infection. In our laboratory serological tests are performed on acute and convalescent (if available) blood specimens for antibody and antigen detection by ELISA, the latter being especially useful when antibiotics have been given before cultures are attempted. The ELISA plates are coated with monoclonal antibody specific for *Y. pestis* F1. The test conformation varies, depending whether antigen capture or antibody detection is to be performed, but it is a straightforward sandwich ELISA technique using peroxidase-labelled anti-immunoglobulin second-step antibodies for visualisation or quantitation by spectrophotometer (WILLIAMS *et al.*, 1986, 1988). Other serological techniques, such as complement fixation, immunofluorescence, agar gel precipitation, latex agglutination, and radioimmunoassay, have been applied to plague diagnosis (ISACSON, 1984). A new approach to F1 antigen detection is a fibre optic biosensor which is claimed to rapidly and safely detect fluorescence of bound immune complexes (CAO *et al.*, 1995).

Molecular methods

Rapid detection of *Y. pestis* by DNA hybridisation is possible if suitable gene sequences such as those encoding species-specific virulence factors can be targeted (GEMSKI *et al.*, 1987; THOMAS *et al.*, 1990). Oligonucleotide probe hybridisation can demonstrate presence of *Y. pestis* in fleas but 10^5-10^6 organisms are needed needed to give reliable results (MCDONOUGH *et al.*, 1988). Molecular typing of *Y. pestis* isolates is used elucidate the epidemiology of present and past plague. Ribotyping, pulse field gel electrophoresis, repetitive sequence analysis, and plasmid restriction profile analysis have been or are currently being applied to *Y. pestis* (GUIYOULE *et al.*, 1994; PRENTICE & CARNIEL, 1995; personal communication, E. Carniel, Institut Pasteur, Paris).

Polymerase chain reaction

Amongst molecular techniques, the polymerase chain reaction (PCR) is an attractive method for detection of *Y. pestis* because it is rapid, highly specific and sensitive, and does not need pure cultures or radioactive reagents. A number of applications of the technique to plague diagnosis or surveillance have been described (CAMPBELL *et al.*, 1993; HINNEBUSCH & SCHWAN, 1993; NORKINA *et al.*, 1994). In our laboratory we are applying

nested PCR to environmental samples in order to study the ecology of *Y. pestis* during and between epidemics (unpublished). One possible disadvantage of PCR is that the viability of the target bacteria cannot be established. As few as 10 colony forming units of the organism can be detected, but as with other applications of the technique, PCR is susceptible to contamination and false positive results. Special precautions are needed to minimise these problems, which may put it out of reach of most routine diagnostic laboratories.

Chromatographic assay

This type of assay can detect *Y. pestis* F1 antigen, and anti-plague IgM and IgG within a short period. A current version is a one step hand-held assay (personal communication, Dr J. Burans, Naval Medical Research Institute, Bethesda, MD). Serum or homogenized sputum is added to the end of a strip on which the stabilised test reagents are present; the sample diffuses along the strip and after 10 minutes the test can be read. For antibody detection, colloidal gold-labelled anti-human IgM or IgG combines with IgM or IgG anti-F1 antibody in the specimen, forming a complex. The complex will combine with the F1 antigen present in the assay strip and produce a visible line. The plague F1 antigen capture chromatographic assay uses a colloidal gold marker attached to detector antibody (anti-F1 monoclonal antibody). This is present in the assay strip and will combine with the capture antibody (rabbit anti-*Y. pestis),* and a complex forms resulting in a visible line. This rapid test holds great promise for use in areas where laboratory facilities are limited, and the results of field trials are awaited with interest.

DISCUSSION

All the identification methods discussed have advantages and disadvantages and some are better suited to certain applications than others. Plague epidemiology, public health resources, and the standard of laboratory facilities vary so much across the plague endemic areas of the world that it is difficult to generalise about the utility of any particular test, apart from emphasizing the importance of basic staining and culture techniques, in conjunction with clinical assesment, in human cases. It is necessary to confirm suspected cultures or specimens by full bacteriological examination. Direct immunofluorescence of bubo aspirate, although a rapid test, has led to a missed plague diagnosis when reported negative, with a fatal outcome (CROOK & TEMPEST, 1992). The problems surrounding the diagnosis of plague during the recent epidemic in India (DAR *et al.*, 1994; KUMAR, 1995) show that clinical laboratory diagnosis is not always optimal, even in countries with a long history of plague. On the surveillance side, there are many techniques available and the local situation and resources will determine which should be used (BAHMANYAR & CAVANAUGH, 1976). The basic surveillance methods are aimed at identifying and enumerating potential mammalian host and flea vector species, and demonstrating plague infections in order to anticipate epizootics before they spill over into human populations. Experience has shown that methods using the capture and bacteriological examination of rodents rarely yield positive results except during actual epizootics. SHEPHERD & LEMAN

(1985) failed to culture *Y. pestis* from any of 4516 rodents of 27 species collected during surveillance activities in South Africa. Serological methods can be used to detect the presence of plague, but when applied to susceptible rodents in plague foci usually yield few positives, mirroring bacteriological results. These species do not survive to carry *Y. pestis* nor develop antibodies, making them unsuitable for monitoring purposes. On the other hand, RUST *et al.* (1971) showed that dogs are relatively resistant to plague and develop high antibody titres, ideal attributes of sentinel animals. Seropositivity rates in dogs can be used to accurately identify epicentres and the direction of spread of outbreaks (ISACSON, 1984). The discovery that sibling species of *Mastomys*, an important bridging host in southern African plague ecology, varied in susceptibility to plague (ISACSON *et al.*, 1983) had major implications for plague surveillance, both in southern Africa and elsewhere. Modern molecular techniques will play an ever more important role in plague surveillance, but cheap, simple and robust versions are needed to make them accessible to many laboratories in plague endemic areas. Whatever techniques are used for plague identification, the safety of laboratory workers must be foremost, and specially designed and dedicated facilities, together with adequate staff training, are the ideal.

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LEPTOSPIROSIS IN ANIMALS AND HUMANS IN SELECTED AREAS OF TANZANIA

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Abstract. A serosurvey was carried out in selected areas of Tanzania to determine the prevalence of leptospirosis in animals and humans. Sera of rodents (n=537), cattle (n=374), dogs (n=208) and humans (n=375) were screened for antibodies by microagglutination (MAT) procedure. The areas studied included swampy or irrigated sugar cane and rice fields (Mtibwa-Morogoro, Sangasanga-Morogoro, Lower Moshi), highlands (Moshi Highlands, Lushoto, Mbizi Forests-Rukwa), pastoral plateaus (Singida, Mwanza, Mbeya, Mbinga), and a Lake basin (Lake Rukwa). *Leptospira interrogans* serovars *icterohaemorrhagiae, hardjo, canicola, pyrogenes* and *grippotyphosa* served as reference antigens in the MAT assay. Antibodies to serovar *icterohaemorrhagiae* were demonstrated in 1.9% of the sera of examined rodents (*Mastomys natalensis*, *Rattus rattus*). Cattle sera showed the presence of antibodies to serovars *hardjo* (5.6%) and *pyrogenes* (1.9%) respectively, and dog sera showed antibodies to serovars *icterohaemorrhagiae* (37%), and *canicola* (0.5%) respectively. A single sample of the human sera agglutinated with serovar *grippotyphosa.* In an attempt to isolate leptospires from urine of 1021 cattle at a slaughterhouse in Morogoro, 7 isolates were obtained. This study has shown that leptospirosis is a potential public health hazard in certain areas of Tanzania.

Key words: Leptospirosis, Seroprevalence, Microagglutination, Isolation.

INTRODUCTION

Leptospirosis is a bacterial disease of worldwide distribution, which affects all mammals, including humans, livestock and wildlife (WHO,1967; THIERMAN, 1984). In humans, leptospirosis may present as a hyper-acute disease leading to quick death, but can also show as a mild febrile disease – «pyrexia of uknown origin» (PUO) (TERPSTRA, 1992). In both cases, it is difficult to diagnose leptospirosis clinically, and unless the clinician specifically looks for it, leptospirosis can be easily overlooked or misdiagnosed.

In theory, any mammal can be infected with one or more serovars of *L. interrogans* but certain serovars show some degree of host specificty. Examples of such serovars and their preferential host (in brackets) include serovars *icterohaemorrhagiae* (rodents), *canicola* (dogs), and *hardjo* (cattle) (THIERMAN, 1984; EVERARD, 1992).

Rodents are no doubt the natural reservoir hosts of many leptospiral serovars. In the tropics, peridomestic and field rodents, such as *Rattus rattus* (L., 1758), *Mastomys natalensis* (Smith, 1834), *Cricetomys gambianus* (Waterhouse, 1840) and *Arvicanthis niloticus* (Desmarest, 1822) are known to be the primary reservoirs of leptospires. Terri-

tory marking canivores are probably the second most highly infected animals, and intra and interspecific transmission within infected hosts may persist in an area for a long time, especially if climatic conditions are favourable for the intermittent survival of the leptospires in the environment (EVERARD, 1992).

Leptospirosis has been known for about a century, since WEIL (1886) and INADA (1916) described this disease and its etiological agent for the first time. However, knowledge about the infectious agent and the prevalence of this disease in many tropical countries is limited. Consequently, the epidemiology of this disease in livestock and humans and its impact on public health in these parts of the world is poorly documented and its assessment largely based on speculations (ELLIS, 1984).

In the East African region, animal leptospirosis was first documented in Kenya and Uganda by BALL (1966). Additional reports on leptospirosis from this region were by KRANENDONK *et al.* (1968) who isolated three new serovars (*kanana*, *lambwe* and *njenga*) from the Coastal Province of Kenya. Human infection, also in Kenya, was first reported by FORRESTER *et al.* (1969), and by DE GEUS *et al.* (1977). These reports were complemented by the isolation of the infectious agent and the description of a new serovar (*kibos*) (DIKKEN *et al.*, 1981; KMETY & DIKKEN, 1993).

Serodiagnosis is the most common approach used to survey leptospirosis in environments where the disease has not been previously reported. Over the years, the microagglutination-test (MAT) has been established as the standard assay for the diagnosis of leptospirosis (COLE *et al.*, 1973; KORVER, 1992; FERESU *et al.*, 1995). The MAT is, however, laborious and time consuming and requires maintenance of numerous live reference antigens. It may also fail to detect antibodies to unique serovars in a newly studied area, where reference strains have to be used instead of indigeneous strains. On the other hand, the advantage of MAT is that it can be carried out even in modestly furnished laboratories because it does not require expensive equipment. For the clinician, definitive diagnosis of leptospirosis is the isolation of the infectious microorganism from the host, but isolation is difficult, a lenghthy process, and often unsuccessful even in specialized laboratories (TERPSTRA, 1992).

This paper reports on a study of seroprevalence of leptospirosis in rodents, cattle, dogs, and humans, carried out in selected areas of Tanzania, and on the isolation of leptospires from urine of cattle submitted for slaughter at the Morogoro town slaughterhouse.

MATERIAL AND METHODS

Leptospira reference cultures (antigens)

Reference strains for use in MAT were kindly supplied by the WHO Reference Laboratory at the Royal Tropical Institute of Hygiene (KIT), Amsterdam, Netherlands. The reference strains were maintained at room temperature (20°-27°C) in Fletchers's medium (DIFCO, Laboratories, Detroit, USA) supplemented with leptospira enrichment medium (DIFCO), and 5-fluorouracil (FU) (0.5%). Growth and purity of the cultures was monitored by dark field (DF) microscopy at seven day intervals, and subculturing in fresh

medium was done either when the cultures became too dense, or after 28 days of growth. Contaminated cultures were purified by either subculturing in Ellinghausen-McCullough's medium, as modified by Johnson and Harris (EMJH), supplemented with cyclohexamide (0.5 mg/ml) as selective inhibitor, or by suspending the cultures in EMJH and then filtering into fresh Fletcher's medium through syringe adaptable 0.22m filters (Millipore Corporation, Bedford Ma. USA).

Collection of test sera

Rodents (n=537) were trapped from swampy or irrigated agricultural areas with sugar cane, and rice farms (Mtibwa-Morogoro, Sangasanga-Morogoro, and Lower Moshi); from highland valleys and tropical forests (Lushoto, and Mbizi Forest-Rukwa respectively), and from the Singida Plateau (Fig. 1). Retroorbital blood was collected from these rodents and the sera were separated and stored at -20° C until tested. All rodent sera were tested against serovar *icterohaemorrhagiae*.

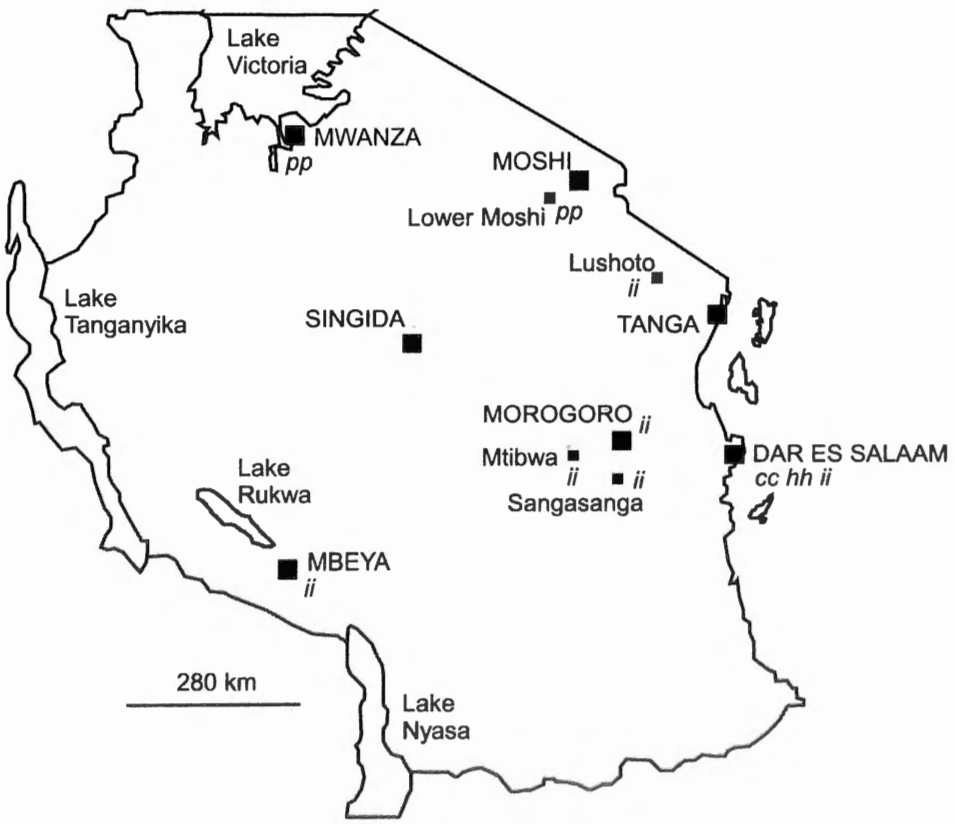

Fig. 1. – Map of Tanzania showing areas of seroprevalence for leptospirosis. cc=canicola; hh=hardjo; ii=icterohaemorrhagiae; pp=pyrogenes.

Cattle were sampled by taking jugular blood of 374 animals originating from dairy farms and ranches in Lower Moshi and Moshi highlands respectively, and from pastoral plains (Iringa, Mbeya, Mbinga-Ruvuma, and Mwanza). Samples were also taken in the Dar es Salaam urban area (Fig. 1). All cattle sera were tested against serovar *hardjo*, and then 360 of these samples were tested against serovar *pyrogenes*.

Dogs (n=208) were sampled by venipuncture of the saphenum veins. Of these, 39 were domestic, unrestrained dogs from different villages in the Lower Moshi area, and 18 were from villages in the Singida plateau. The rest of the dogs had been brought in for dipping at various veterinary clinics in Dar es Salaam town (73 dogs), and at the Sokoine University Veterinary Clinic, Morogoro (70 dogs). The sera were first tested against serovar *canicola*, and in a second run, the sera from Singida were excluded and the remaining were tested against serovar *icterohaemorrhagiae*.

Human sera were donated by 159 young adult male cane cutters from Kilombero Sugar Company (Fig. 1) and another 216 sera were kindly provided by Dr. J. Shao of the Department of Microbiology, Muhimbili University College of Health Sciences (MUCHS), Dar es Salaam. These sera were screened against serovars *icterohaemorrhagiae* and *grippotyphosa*. None of the domestic animals and humans showed any clinical signs suggesting leptospirosis.

Antibody detection

All sera were screened for antibodies against leptospires using MAT as described by COLE *et al.* (1973). Briefly, the sera (100µl amounts) were titrated with phosphate buffered saline (PBS) in `U' microtitration plates to obtain an initial titre range of 1:20-1:160. Equal volumes of antigens grown in liquid EMJH medium to a density of approximately 3×10^8 leptospires/ml on the MacFarland scale were then added and the plates incubated at room temperature for 2 h. The reactions were then examined for agglutination by DF microsocopy. A serum was considered positive if 50% or more of the microorganisms in the microtiter well agglutinated at titre 1:160. These positive sera were subsequently titrated to 1:20480 in PBS and tested again with the antigen to establish the antibody titre. Control positive and negative sera were supplied by DIFCO Laboratories, Detroit, and KIT, Amsterdam, Netherlands, respectively.

Isolation of leptospires

Urine samples were collected from freshly excised bladders of 1021 clinically healthy cattle at the Morogoro abbatoir. Urine samples (20 µl) were examined for spirochetes by DF microscopy, and quantities of 5 ml were inoculated in McCartney bottles containing 15 ml Fletcher's medium with 10% leptospira enrichment. These cultures were then incubated (30° C) with shaking for 28 days and examined for leptospira growth at 7 day intervals.

RESULTS

The seropositivity to different leptospira serovars per species and locality are shown in Table 1.

TABLE 1

Seropositivity for leptospirosis in animals and humans in Tanzania

Sampled group	*N positive/N tested*	*% Positive*	*Serovar*	*Locality*
Rodents	10/537	1.8	*icterohaemorrhagiae*	Sangasanga, Mtibwa, Lushoto
Cattle	21/374	5.6	*hardjo*	Lower Moshi, Dar es Salaam, Mbeya
Cattle	7/360	1.9	*pyrogenes*	Mwanza, Mbeya
Dogs	79/208	38	*icterohaemorrhagiae*	Lower Moshi, Morogoro
Dogs	1/208	0.5	*canicola*	Dar es Salaam
Humans	1/375	0.3	*grippotyphosa*	MUCHS – Dar es Salaam

Seroprevalence in rodents

Seropositivity to serovar *icterohaemorrhagiae* was recorded with 10 out of 537 (1.9%) rodent sera examined. The positive sera, (number in parentheses) originated from Mtibwa-Morogoro (5), Sangasanga-Morogoro (3) and Lushoto (2) (Fig. 1). Of these sera, 7 were from *Mastomys natalensis,* and 3 were from *Rattus rattus*.

Seroprevalence in cattle

Out of 374 cattle sera screened, 21 (5.6%) agglutinated against serovar *hardjo,* and 7 (1.9)% agglutinated against serovar *pyrogenes* (Tab. 1). Fourteen of the *hardjo*-positive sera were from Lower Moshi, 2 from Dar es Salaam town, 2 from Mbeya, and 3 from Mwanza. Three of the *pyrogenes*-positive sera were from Mwanza and 4 from Mbeya (Fig. 1).

Seroprevalence in dogs

Out of 208 dog sera screened against serovar *icterohaemorrhagiae,* 79 samples (38%) reacted positively. These were from Lower Moshi (23 samples), Dar es Salaam town (29 samples), and Morogoro town (27 samples). Only one out of 208 dog serum samples (0.5%) agglutinated against serovar *canicola*. This serum originated from Dar-es-Salaam town (Fig. 1).

Seroprevalence in humans.

Out of the 375 human serum samples, one (0.3%) reacted positively to serovar *grippotyphosa*. This sample was obtained from MUCHS, Dar es Salaam. None of the human sera reacted against serovar *icterohaemorrhagiae*.

Isolation of leptospires from cattle urine.

Out of 1021 cattle urine samples screened, 7 were positive for leptospires. The microorganisms were isolated and purified in Fletcher's and EMJH media, for subsequent characterization.

DISCUSSION

This is the first report on leptospirosis in Tanzania. Although the study focused on relatively few areas, it has provided substantial evidence of leptospiral prevalence in rodents, cattle, dogs and humans in Tanzania. The prevalence of serovar *icterohaemorrhagiae* in rodents was demonstrated with two species (*M. natalensis* and *R. rattus*) in the Morogoro area. These two species are the most abundant small mammals in Tanzania, and could therefore be the most important rodent reservoirs of leptospires in Tanzania. At least *M. natalensis* has been shown to be a potential carrier of other serious diseases in Tanzania (LEIRS *et al.*, 1988; KILONZO *et al.*, 1992).

The overall highest seroprevalence (38%) was demonstrated with serovar *icterohaemorrhagiae* in dogs in the Lower Moshi area; however, this serovar was not prevalent in the dogs from the other areas studied. Dogs may, therefore, be important maintenance hosts of serovar *icterohaemorrhagiae* in the Lower Moshi area. It is in agreement with previous reports that canines are, next to rodents, the most common carriers of leptospires, and that the dog is a potential transmitter of leptospirosis to humans in the domestic environment (EVERARD, 1992). Seroprevalence in humans in the Lower Moshi area was not studied.

The observed high seroprevalence of leptospirosis in dogs in Lower Moshi could be due to intraspecific transmission among dogs because in this area dog keeping is common; at the same time, dogs are never restrained and therefore are able to stray in the neighbourhood.

The seroprevalence of serovar *hardjo* in cattle is in agreement with previous reports that this serovar is frequently found in bovines (FERESU, 1987). In this study, serovar *hardjo* appeared uncommon in cattle in the pastoral plain (Mwanza), where serovar *pyrogenes* was the prevalent one. Apparently certain serovars might be common in some areas of Tanzania but not in others.

Seropositivity in humans was demonstrated with only one serum sample against serovar *grippotyphosa*. This prevalence can be considered an underestimate because of the limited number of sera and the serovars tested. Additional studies on humans are desirable, particularly in the Lower Moshi area, to see whether there is a correlation between the high seroprevalence recorded with dogs, and human infection.

The isolation of 7 leptospires from 1021 cattle urine complemented the serological findings. However, this isolation (0.7%) may not be a true reflection of the number of cattle infected because the rate of success in isolation of leptospires from urine is relatively low (FAINE, 1982). Additional studies involving isolations from kidneys could give a clearer prevalence rate in the cattle.

The seroprevalence and the isolation of leptospires demonstrated in this study came as no surprise to us because the environment in many parts of Tanzania is favourable for the survival of leptospires. Such environment includes the sugar cane and rice plantations, which are in marshy or irrigated lands; and flood basins, where fishing and livestock grazing are commonly practiced. Also, many natural reservoirs and maintenance hosts of leptospires are found in all these areas. The existence of reports on the prevalence of leptospirosis in livestock and humans in the neighbouring countries of Kenya, Uganda, and Zimbabwe further supports our findings for Tanzania.

Leptospirosis is a health hazard that has been overlooked in Tanzania and elsewhere in Africa (ELLIS, 1984; MACHANG'U 1992). There is a need to alert all occupational groups at risk of the dangers of infection. Public health professionals, veterinarians, and physicians should consider including leptospirosis in the diagnoses of all clinical PUO cases of animals and humans, and in other conditions with symptoms suggesting leptospirosis. By doing so, the extent of leptospirosis and its economic importance will be known and preventive approaches could be initiated.

Future studies will aim at further isolation and characterization of endemic serovars, and at establishing the epidemiological patterns of leptospirosis in different parts of Tanzania.

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DOMESTIC RODENTS AS RESERVOIRS OF PATHOGENIC *LEPTOSPIRA* ON TWO CITY OF HARARE FARMS: PRELIMINARY RESULTS OF BACTERIOLOGICAL AND SEROLOGICAL STUDIES

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Abstract. Bacteriological and serological studies were carried out to determine the role domestic rodents play in transmitting leptospirosis on two City of Harare farms. Rodents were trapped and their kidneys and urine cultured for *Leptospira*. The rodents and volunteer blood donors, from farm workers and their families, were bled and the sera screened for antibodies against representative strains of eight serogroups of *Leptospira* using the Microscopic Agglutination Test.

Rattus rattus was the most abundant rodent caught and yielded the majority of the *Leptospira* isolates. The prevalence of leptospiral titres at a serum dilution of 1:100, was 62.5% for the rodents and 82% for the volunteer blood donors. The most common titres in both the rodents and humans were to antigens from the Icterohaemorrhagiae, Pyrogenes and Grippotyphosa serogroups. The results suggest that leptospirosis is a common occupational disease of workers on the two farms which is transmitted to them by rodents.

Key words: rodents, reservoirs, *Leptospira*.

INTRODUCTION

Leptospirosis is an important zoonosis which affects all mammals and can be transmitted among domestic, wild animals and man. The clinical picture in man may vary considerably, from mild illness resembling flue, to severe illness as in Weil's disease where there is renal failure and meningitis. Death from leptospirosis may occur in 5-30% of untreated human cases (WAITKINS, 1983).

Rodents living in close association with humans have been found to be the most important reservoirs of leptospires (FAINE, 1982). They then transmit the leptospires to man either directly or indirectly through an intermediary domestic animal species. Reservoir rodents maintain the organism in their kidneys and pass it to humans by excreting urine in homes, paddocks, water-ponds, sewers and on farms where humans work or live. Therefore although leptospirosis may not be ranked as one of the most important dis-

eases universally, it can be very common in certain occupational groups such as agricultural workers, refuse collectors, sewer and sewage workers who are exposed to environments frequented by these rodents (WAITKINS, 1983). The principal ports of entry of the leptospires are: the skin especially when it has been wounded, abraded or softened by continued contact with water; and the mucous membranes of the eyes, nose and throat (FAINE, 1982).

Leptospirosis is known to be more prevalent in tropical and subtropical regions, particularly in the communal areas and on farms, due to the low standard of living, labour intensive agricultural practices, limited sanitation facilities and poor animal handling techniques (FERESU, 1990). Most people living in these communities are usually bare-foot and may be exposed to leptospires from the rodents in pastures, ponds and rivers. Their children often play and swim in areas frequented by rodents.

Research on leptospirosis has been very limited on the African continent, but the few studies done have demonstrated that it exists in all the countries where studies have been undertaken (DIALLO & DENNIS, 1982). In Zimbabwe, although evidence of leptospirosis was established as early as 1956, there have been very few documented reports, most of which were entirely based on clinical diagnosis (MOSSOP, 1974; WILES, 1979). There have also been limited serological surveys in humans (GRAF, 1965), in dogs (BANKS & PIGOTT, 1979) and two large surveys in cattle (SWANEPOEL *et al.*, 1975; FERESU, 1987). A few isolation studies have resulted in the discovery of five new serovars of *Leptospira*: zimbabwe; mombe; mhou; marondera and proposed ngavi (FERESU *et al.*, 1993; 1994; 1996; FERESU, unpublished results).

The purpose of the current study was to investigate the role of rodents in transmitting leptospirosis to humans on two City of Harare farms using both cultural and serological tests, since a strain of *Leptospira* had previously been isolated from a rat on one of the farms (Central Veterinary Research Laboratory Reports, 1978).

MATERIAL AND METHODS

The study areas

The Harare Municipality owns several farms at the periphery of the City. It uses these farms to purify sewage effluent whose quality cannot be discharged directly into the river system, by flood irrigating the pastures and allowing the water to be filtered naturally by the ground. The green pastures are used for cattle ranching.

The two farms are situated 30 km south west of the University of Zimbabwe. At the time of the study, Farm A had 67 while farm B had 41 permanent workers. The workers were allowed to stay with their families, bringing the total populations of the farms to 350 and 250 respectively. Each household was provided with a decent brick main house and was allowed to construct a pole and dagga hut to use as a kitchen. The quality of these kitchens was not controlled, making them very varied, with some being quite poor and allowing for heavy infestation with rodents.

Each household was allocated a piece of land for gardening which was also flood irrigated with sewage effluent. Thus the poor kitchens and potential extensive exposure of the farm workers and their families to flood irrigated pastures and gardens, presented with conditions conducive to the transmission of leptospirosis.

Trapping of rodents

The rodents of interest were *Rattus rattus* (L., 1758), *Mastomys* sp., *Mus musculus* L., 1758, *Rhabdomys pumilio* (Sparrmann, 1784), *Otomys angoniensis* Wroughton, 1906 and *Aethomys chrysophilus* (de Winton, 1897). They were trapped in and around human dwellings, in gardens and in storage sheds on the two farms over a period of approximately two years (January 1995 to October 1996), encompassing two wet and two dry seasons. The rodents were identified using morphological characters such as size and shape of body, colour of fur, length of the head in relation to the tail, size of the rear feet, relative size of the ears and eyes, and shape of droppings (GWINNER *et al.*, 1990). The identifications were verified by the National Museums of Zimbabwe.

Each rodent species was further grouped according to sex and age. Only two age groups were considered, the juveniles and the old, the latter having live weights (grammes) in excess of: 10, *M. musculus;* 20, *R. pumilio;* 25, *Mastomys* sp.; 50, *A. chrysophilus*; and 120, for both *R. rattus* and *O. angoniensis* (GWINNER *et al.*, 1990).

Isolation studies

Urine from the trapped rodents was collected directly by sterile bladder tapping or by flushing the bladder with 1 ml of 1% bovine serum albumin (BSA) in phosphate buffered saline (PBS), pH 7.2. Whole kidneys were aseptically placed in a sterile plastic stomacher bag containing 10 ml of 1% BSA in PBS and homogenized. The urine and kidney homogenates were then serially diluted (10^{-1} through 10^{-4}) in 1% BSA in PBS. From each dilution, 1 ml was inoculated into a tube containing 9 ml of semi-solid modified Ellinghausen and McCullough (EMJH) medium (JOHNSON & HARRISON, 1967) with 5-fluorouracil (100 mg/ml) added. The tubes were incubated at 29°C for 12 weeks and checked weekly for characteristic leptospiral growth by darkfield microscopy.

Bleeding

Rodents. Blood from the trapped rodents was collected directly from the abdominal aorta. The sera were stored at -20°C until tested.

Farm workers. Volunteer blood donors were solicited among the farm workers and members of their families. Each volunteer was bled four times (at the beginning of each season) to determine their serological status during the course of the study. The sera were stored at -20°C until tested.

Questionnaire. A questionnaire was administered to the donors at the time of the third blood collection. The questions asked related to sex, age, period of stay on the farm, occu-

pational duties, illness with leptospirosis related symptoms during the course of the study and the level of exposure to contaminated environments.

Serological studies

Both the rodent and human sera were screened for leptospiral antibodies using the microscopic agglutination test (COLE *et al.*, 1973) at 1: 100 dilution. The antigens used included 13 representative strains of eight *Leptospira* serogroups: Icterohaemorrhagiae (strains RGA & SBF 23); Pyrogenes (SBF 20); Grippotyphosa (SBF 32 & Moskva); Australis (SBF 3 & Jez brat); Mini (Sari); Ballum (Mus 127); Hebdomadis (SBF 5, SBF 40) and Tarassovi (SBF 16, Perepelitsin). All SBF strains are Zimbabwean strains which were included to enhance positivity as more and higher titre reactions are often observed when indigenous strains are used rather than exotic reference strains (FAINE, 1982).

A serum was considered positive when 50% or more of the leptospires agglutinated.

Malaria. The human blood was also examined for malaria parasites after staining with the Giemsa stain. This was done to exclude malaria as it presents with symptoms similar to those of leptospirosis.

RESULTS

Trapping

The rodent species trapped over the four seasons are presented in Table 1. *Rattus rattus* and *Mastomys* sp. were the most abundant species caught throughout the trapping period. A large number of *R. pumilio* were also caught with most of them being trapped during the first dry season. *Aethomys chrysophilus*, *O. angoniensis* and *M. musculus* were rare and seasonal.

TABLE 1

Species distribution and numbers of rodents trapped at the two City of Harare farms during the four seasons

Rodent species	*Seasons*				
	1st Wet	*1st Dry*	*2nd Wet*	*2nd Dry*	*Total*
R. rattus	55	99	92	47	293
Mastomys sp.	6	44	21	14	85
R. pumilio	4	32	0	8	44
M. musculus	0	0	3	0	3
A. chrysophilus	0	0	4	1	5
O. angoniensis	0	5	0	2	7
Total	*65*	*180*	*120*	*72*	*437*

Isolation studies

A total of 52 strains were isolated from the rodents with most (46) being obtained from *R. rattus* (Table 2). More isolates (41) were obtained during the second wet season than during the first wet (5) and dry seasons (each 3). Forty of these isolates were from kidneys while the remaining 12 were from urine. Thus 12 rodents had isolates from both kidneys and urine, making the overall isolation rate 9%.

TABLE 2

Number of leptospiral isolates from each rodent species, overall % sero-positivity and sero-positivity of the sera within each rodent species

Rodent species	*Number trapped*	*Number of isolates*	*Overall % sero-positivity*	*% sero-positivity within species*
R. rattus	293	46	49	73
Mastomys sp.	85	2	7	35
R. pumilio	44	0	6	57
M. musculus	3	4	0.5	67
A. chrysophilus	5	0	0	0
O. angoniensis	7	0	0	0
Total	*437*	*52*	*62.5*	-

- = non-applicable.

Serological studies

Rodents. A large number, 62.5%, of the rodents had titres against one or more of the 13 *Leptospira* antigens. High percentages of *R. rattus, Mastomys* sp., *R. pumilio* and *M. musculus* had titres to the leptospiral antigens (Table 2). All the *O. angoniensis* and *A. chrysophilus* trapped were sero-negative.

More adults than juveniles were sero-positive. Sero-positivity was, however, not dependant on sex or season. Most rodent sera were positive against representative antigens of serogroups Pyrogenes, Icterohaemorrhagiae and Grippotyphosa (Table 3).

Blood donors. A total of 182 volunteer blood donors was initially recruited (102 from Farm A and 80 from Farm B) but 27 dropped out during the course of the study. One hundred and fifty (82%) volunteers had positive titres against one or more *Leptospira* antigens for at least one of their four bleeds. There was no particular trend in sero-positivity of the blood donors over seasons, although the percentage sero-positivity remained quite high over the whole sampling period.

The reactions with the different antigens were similar to those observed for rodents, with the most common titres being against representative strains of serogroups Icterohaemorrhagiae, Pyrogenes and Grippotyphosa (Table 3). This implies possible trans-

mission of leptospirosis from rodents to the volunteer farm workers and members of their families.

TABLE 3

Comparison of % sero-positivity to the various Leptospira *serogroups between human and rodent sera*

Antigen	*% sero-positive*	
	Rodent sera	*Human sera*
Pyrogenes	40	66
Icterohaemorrhagiae	22	69
Grippotyphosa	17	43
Australis	12	15
Mini	10	16
Autumnalis	8.5	21
Javanica	8	10
Ballum	6	8
Hebdomadis	3	13
Tarassovi	2	12

Questionnaire responses. People who had stayed on the farm for over 10 years were more sero-positive (51%) than those who had stayed for shorter periods (43%). More family members who donated blood (64%) were sero-positive than the volunteer municipal employees (34%). Most of the sero-positive donors remembered having suffered from leptospirosis related illnesses such as fever (84%), chills (67%) and haemorrhages (38%) during the study period. From their responses, 99% of the sero-positive volunteers had been exposed to the effluent used to flood irrigate the pastures and gardens and in most cases (85%) contact had been made with the effluent without protective clothing.

Malaria. No malaria parasites were detected in any of the blood samples.

DISCUSSION

This survey is the first comprehensive study to determine the role domestic rodents play as maintenance hosts of *Leptospira* and to demonstrate leptospirosis as a possible occupational disease of farm workers in Zimbabwe.

The results indicate *R. rattus* as the most abundant and important carrier of *Leptospira* on two City of Harare farms. *Rattus rattus* is one of the ubiquitous rodent species known to maintain leptospires throughout the world, and as few as two shedder rats per hectare can maintain leptospirosis within a population and cause epidemics in farm workers (FAINE, 1982).

Although the isolates have not yet been identified, the serological studies indicate that, in Zimbabwe, domestic rodents may be important reservoirs and transmitters of strains of serogroups Pyrogenes, Icterohaemorrhagiae and Grippotyphosa to humans. Transmission of strains of similar serogroups by domestic rodents has been observed elsewhere on the African continent, with rats having been shown to be carriers of serovar icterohaemorrhagiae in Egypt (McGuire & Myers, 1957); Tunisia (Bakoss & Chadli, 1965) and South Africa (Herr *et al.*, 1982), while serovar grippotyphosa has been isolated from *Mus musculus* in Egypt (Barsoum *et al.*, 1973).

Our results can however only be viewed as being suggestive as other possible sources of leptospiral infection and transmission on the farms were not investigated. Strains of *Leptospira* in municipal raw sewage, have been known to survive sewage treatment (Jones *et al.*, 1981), thus the effluent used to irrigate the pastures and gardens may have initially contained strains of *Leptospira* from carrier convalescents in the municipal population. Transmission of *Leptospira* from cattle is also possible as strains of the three serogroups have been isolated from Zimbabwe beef cattle (Feresu, 1990).

The results indicate that leptospirosis is a common occupational disease of farm workers and members of their families as the majority of the blood donors had antibodies against *Leptospira* and recalled having suffered from leptospirosis related symptoms. In Zimbabwe leptospirosis is not commonly included in differential diagnosis as it presents with symptoms similar to the more well known parasitic, viral and other bacterial infections such as malaria, hepatitis, rickettsia, Rift Valley fever and brucellosis. No malarial parasites were demonstrated in the blood of our study subjects. Thus the results of this study suggest that leptospirosis should be included in differential diagnosis, particularly for those patients from high risk occupational groups.

The higher numbers of family members with antibodies to *Leptospira* antigens may be explained by the practice on the farms where the municipal employees are supplied with protective clothing during working hours while the family members usually work barefoot in the flood irrigated gardens, children enjoy playing in mud and women cook and spend more time in rodent infested kitchens. However, although the municipal employees used protective clothing, their level of sero-positivity was still high (34%), because the wearing of gloves maybe impracticable when performing some chores, cattle may splash the effluent during handling and the effluent can seep in boots making them more hazardous than protective (Faine, 1982).

The present study has once again demonstrated the presence of strains of the genus *Leptospira* on the African continent and the need to carry out further research to determine the prevalence and importance of leptospirosis on the continent.

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A PRELIMINARY SURVEY OF MACROPARASITE COMMUNITIES OF RODENTS OF KAHAWA, CENTRAL KENYA

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Abstract. A preliminary survey of macroparasite communities of *Mastomys natalensis, Lemniscomys striatus, Arvicanthis niloticus, Tatera robusta* and *Mus minutoides* trapped alive in three microhabitat types within a dry sub-humid grassland showed a high diversity in both ecto- and endoparasites. *Rhipicephalus appendiculatus, Amblyomma variegatum, Boophilus* sp., *Orchopeas* sp., *Leptopsylla* sp. and *Xenopsylla cheopis* were collected from the skin and hair. *Fasciola* sp., *Taenia* sp., *Hymenolepis* sp., *Ascaris* sp., *Trichostrongyus* sp., *Clonorchis* sp. and *Heterophyes* sp. were retrieved from the alimentary canal or their eggs isolated from faeces. The distribution of rodent macroparasites was dependent on host species and microhabitat. Mean ectoparasite intensity also varied with host microhabitat and species. *Arvicanthis niloticus* had heavier intestinal macroparasite infestation compared with *Mastomys natalensis* and *Lemniscomys striatus*. Generally, bigger hosts exhibited a heavier parasitic load. It is inferred that rodents may be important in the transmission and sustenance of livestock diseases in this grassland.

Key words: *Mastomys, Lemniscomys, Arvicanthis*, macroparasites.

INTRODUCTION

Rodent control programmes in sub-Saharan Africa are usually directed at field and storage pest species. However, most rodent pests are also carriers of pathogens (FIEDLER, 1994), which calls for extended studies on their role in transmission or sustenance of human (KILONZO *et al.*, 1992; IKEH *et al.*, 1995; GITHURE *et al.*, 1996), livestock and wildlife (GROBLER *et al.*, 1995) disease. Future rodent management programmes will need to incorporate control of pathogens (microparasites) and their vectors (macroparasites). Studying parasite communities of rodents has also its own ecological significance (POULIN, 1995). In a preliminary study on macroparasites of rodents in three microhabitat types we surveyed diversity, distribution and host factors affecting parasite community composition around Kahawa, Kenya.

MATERIAL AND METHODS

Study area

The study site was located at the Kenyatta University campus in Kahawa, 25 Km north east of Nairobi (1° 14' S, 36° 48' E). The climate there is dry subhumid with an annual temperature range of 20-25°C and rainfall of 600-1100 mm. Three microhabitats were selected, based on vegetation characteristics and anthropogenic activities: (a) an acacia-themeda grassland characterised by *Themeda triandra* Forsk and *Hyparrhenia* spp. with sparse trees and tree-saplings of *Acacia* species; (b) a maize field with post-harvest stalks and *Bidens pilosa* L. with occasional *Leonotis* species; (c) areas with intense human activities, *i.e.* small-livestock pens around University laboratories.

Collection of specimens

Rodents were live-trapped using Sherman traps. For each microhabitat, five traps were checked on two consecutive nights per week for eight weeks in January and April 1996. Peanut butter on commercial mouse-pellets was used for baiting. Trapped animals were transferred to the laboratory, killed with chloroform, and species, sex and body weight were recorded. Each animal was then carefully examined for ectoparasites. Parasites were collected and preserved in 10% buffered formalin before identification. Total number of parasite types and sites of recovery were noted per host specimen. Dissection was carried out and gastrointestinal tracts carefully examined for helminths. Autopsy from lungs and liver was collected for histological analysis. Faecal samples were obtained from the rectum and examined.

Tissue processing

Lung and liver tissues were processed in paraffin wax before sectioning as described by CULLING (1974). Autopsied tissues were dehydrated in alcohol, de-alcoholated and waxed. Waxed tissues were sectioned at 0.5 (m using a sledge microtome (Leitz Weizlar 1512) and fixed on slides with Meyer's egg albumin, rehydrated and stained. DPX mountant (BDH Limited, Poole, England) was added before microscopic observations.

Faecal processing

The formol-ether concentration method (MANSON-BAHR & BELL, 1987) was used to process faecal samples. This involved centrifuging samples in formol-water and ether at 3000 rpm for one minute (RITCHIE, 1948). Sediments were then spread on slides, giemsa-stained and observed under a microscope.

Statistical analyses

Mean intensity of infection was determined for ectoparasites and helminths retrieved from the alimentary canal by calculating the number of parasites per rodent host (total

number of parasites/total number of host individuals). Prevalence of occurrence was applied for eggs in faeces and determined by the percentage of the host population that was infected. A student's t-test was used to compare parasitic load between the sexes. ANOVA was applied to inspect the effects of host habitat and species on parasitic load. Correlation analysis was used to determine any relationship between host body weight and parasitic loads. Simpson's diversity index ($S=1/\Sigma[p_i]^2$) was used to compare diversity of rodents within the microhabitats and of parasites for various environmental factors (BEGON *et al.*, 1990)

RESULTS

During this study, a total of 29 rodents were trapped belonging to five species (Table 1), i.e. four murids (*Mastomys natalensis* [Smith,1834], *Lemniscomys striatus* L. [1758], *Mus minutoides* Smith [1834], *Arvicanthis niloticus* [Desmarest, 1822]) and a cricetid *Tatera robusta* (Cretzschmar, 1830). Captures were low with a trapping success of 12.9% in 240 nights. More than one individual was captured only in respect of *M. natalensis, L. striatus* and *A. niloticus* . *M.natalensis* was captured in all the microhabitats, *L. striatus* in grassland and maize field and *A. niloticus* mainly around small livestock pens and in grassland.

TABLE 1

Number of rodent specimens captured in different microhabitats (G= grassland, M= maize field, P= around small livestock pens) within Kenyatta University, Kenya in January and April 1996

Rodents	*Number captured and microhabitat types*			
	G	*M*	*P*	*Total*
Mastomys natalensis	3	5	6	14
Lemniscomys striatus	5	2	0	7
Arvicanthis niloticus	1	0	5	6
Mus minutoides	0	1	0	1
Tatera robusta	0	0	1	1
Total	9	8	12	29

Macroparasite distribution varied with host microhabitat, species or both (Table 2). Diversity of ectoparasites varied with host factors such as species, habitat and size (Table 3). Similarly, mean intensity of infection of ectoparasites varied significantly with host species ($P<0.05$) and microhabitat ($p<0.01$) (Table 3). Generally, a weak ($r=0.44$) but significant ($p<0.03$, $n=25$) relationship between ectoparasite load and host body size was recorded (Fig. 1). Host parameters affected helminth distribution and *A.niloticus* exhibited significantly ($p<0.05$) heavier loads (Table 3). From the histological preparations, only two individuals showed lesions in lungs (a *L. striatus*) and liver (a *T. robusta).*

TABLE 2

Distribution of rodent macroparasites in relation to host species and micohabitats around Kahawa, Kenya in January and April 1996. The figures show number of specimens from which parasites or helminth eggs were retrieved. Sample sizes are denoted by (n). Rodents were captured in grassland (G), maize fields (M) and around small-livestock pens (P)

Macroparasites	*M. natalensis*			*L. striatus*		*A. niloticus*	
	G	*M*	*P*	*G*	*M*	*G*	*P*
(n)	*3*	*5*	*6*	*5*	*2*	*1*	*5*
Rhipicephalus appendiculatus Neumann, 1901	3	5	4	4	2	1	0
Amblyomma variegatum Fabricius, 1794	0	2	0	2	0	1	0
Boophilus sp.	1	1	0	0	1	0	0
Orchopeas sp.	0	0	2	0	1	0	0
Leptopsylla sp.	0	2	0	4	0	1	0
Xenopsylla cheopis (Rothschild, 1903)	0	2	0	0	0	0	0
Schistosoma spp.	2	4	2	0	0	1	0
Fasciola sp.	1	5	4	2	2	0	0
Taenia spp.	2	2	2	5	1	0	2
Hymenolepis sp.	0	0	0	0	0	0	1
Ascaris sp.	0	1	3	5	2	0	3
Trichostrongyus sp.?	0	0	0	0	1	0	0
Clonorchis sp.?	0	1	0	0	0	0	0
Heterophyes sp.?	0	1	1	0	0	0	0

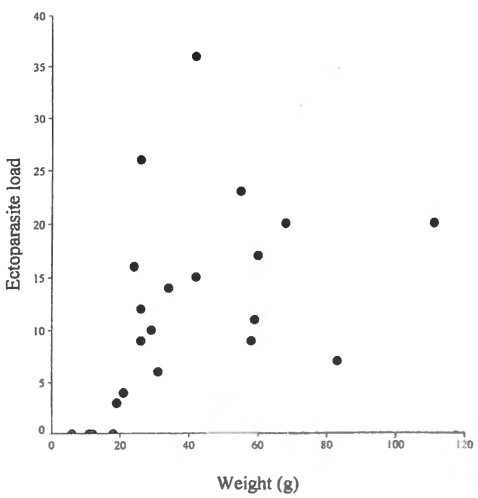

Fig. 1. – A scattergram showing relationship between host weight and ectoparasite loads among rodents (n=25) in Kahawa, Kenya.

TABLE 3

Diversity indices (a) and % mean intensity of infection (b) of macroparasites in relation to various host parameters, i.e. species, microhabitat and size. Mn= Mastomys, Ls= Lemniscomys, An= Arvicanthis, G= grassland, M= maize field, P= around small-livestock pens

species	*microhabitat*			*size (g)*		
	Mn	*Ls*	*An*	*M*	*G*	*P*
					<20	*>20*
(a) Diversity indices						
Ectoparasites	0.8	1.2	1.6	1.4	1.5	1.3
0.2	0.8					
Helminths	2.5	1.8	1.4	1.5	1.4	1.9
0.2	0.5					
(b) Mean intensity of infection (%)						
Ectoparasites	10.9^{a}	12.6^{a}	1.2^{b}	10.8^{1}	17.2^{2}	4.8^{1}
Helminths	1.2^{a}	1.0^{a}	5.3^{b}	0.9	1.4	3.1

Different letters (^{a}) and numbers (^{1}) in superscripts across rows denote significantly different means.

DISCUSSION

In our study we retrieved ectoparasites and endoparasites from diverse taxonomic groups from *M. natalensis, L. striatus, A. niloticus, M. minutoides* and *T. robusta*. Despite the low sample sizes, these preliminary findings have shown that macroparasite distribution in rodents around Kahawa depend to a great extent on (i) species and (ii) microhabitat (Table 2). For ectoparasites, the two factors were also important for their diversity and mean intensity (%) on hosts (Table 3). Rodents captured in the maize field showed higher percent infestation ($p<0.01$) than in the other microhabitats. Ectoparasite infracommunity was more diverse in *A. niloticus* while *L. striatus* and *M. natalensis* displayed heavier loads ($p<0.05$). Such variation in the parasitic community composition in different microhabitats and host species has been documented before (reviewed by POULIN, 1995) and is believed to be due to ecological characteristics such as diet. The significant ($p<0.03$) relationship observed between host size and parasitic load (Fig. 1), which is also consistent with other findings (POULIN, 1995), may be explained by the fact that bigger host animals generally have larger home ranges and presumably thereforeare more exposed to infective stages of parasites.

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EVALUATION OF SYSTEMIC INSECTICIDES MIXED IN RODENTICIDE BAITS FOR PLAGUE VECTOR CONTROL

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Abstract. Rodenticide baits containing systemic insecticides were evaluated in the laboratory for their palatability to the house rat *Rattus rattus* and for their toxicity against the oriental rat flea *Xenopsylla cheopis* - both animals are important vectors of plague in Africa. The test bait and a non-poisonous alternative were given to the rats for four days. The evaluation of the effectiveness was based on mortality and poison bait intake in percent of the total consumption. Different concentrations of technical material and different types of encapsulation of the three insecticides phoxim, fenthion and dimethoate were used in the tests. The rodenticide used was 0.005% bromadiolone. For all three insecticides, a reduced intake of the poisonous bait was observed compared with the test of bromadiolone without insecticide. Based on the acceptance of the baits, the dimethoate encapsulated with beef tallow only was considered as the most promising candidate. The formulation was tested on flea infested rats and after four days, a raised flea mortality was observed.

Key words: Rodenticide bait, systemic insecticides, *Rattus rattus*, *Xenopsylla cheopis*.

INTRODUCTION

Several systemic insecticides have been evaluated in field trials for their effectiveness in flea control when mixed into non-poisonous bait given to rodents (*e.g.* MILLER *et al.*, 1977a, 1977b, 1978). Especially organophosphorus compounds have been reported as having a potential. However, an increase in the acceptibility to the rodents would be desirable, as higher concentrations of insecticides may be used. For this purpose, (micro-)encapsulated formulations of the insecticides could be suitable.

The objective of the present study was to develop rodent baits containing systemic insecticides along with the rodenticide for plague vector control. In the present paper we report on laboratory experiments to 1) evaluate potential insecticides in palatability tests with groups of rats (*Rattus norvegicus* [Berkenhout, 1769] and *Rattus rattus* [L.,1758]) and 2) evaluate the efficacy of these insecticides in the baits on the flea *Xenopsylla cheopis* (Rothschild, 1903) occurring on *Rattus rattus*.

MATERIAL AND METHODS

Rodenticide

The rodenticide chosen for the study was the anticoagulant bromadiolone. Bromadiolone was chosen because it is palatable to many rodent species and because it is being used worldwide (MARSH, 1977; BUCKLE, 1994). A concentration of 0.005% bromadiolone in the bait formulation was used as it is the standard concentration for practical control purposes (BUCKLE, 1994). Bromadiolone is a slow-acting anticoagulant and although one single feeding may be enough for the rodent to ingest a lethal dose, several days will pass before the rodent starts showing symptoms and dies (thus there is time enough for the fleas to die before the death of the rat).

Insecticide

The insecticides and concentrations used in the trials reported here were 0.24% phoxim, 0.15% and 0.3% fenthion and 0.6% dimethoate.

To test whether the acceptability of these compounds to the rodents could be increased, different encapsulated formulations were also used. A commercially available microencapsulated fenthion product (Baytex ME 35%) was tested along with the following formulations made specifically for this project: fenthion and phoxim coated with hydrogenated beef tallow, dicalcium phosphate dihydrate, silicium dioxide and PVP 30 (formulation C), and two formulations of dimethoate - one having a coating of hydrogenated beef tallow alone (formulation X) and another with a coating of hydrogenated beef tallow, dicalcium phosphate dihydrate and PVP 30 (formulation Y). The encapsulated formulations were tested in the concentrations of 0.15% fenthion, 0.24% phoxim and 0.6% dimethoate, respectively.

Rodents

The rodents used for the experiments were a Danish Pest Infestation Laboratory (DPIL) strain of the brown rat *Rattus norvegicus* and a Tanzanian and a DPIL strain of the roof rat *Rattus rattus*. (The Tanzanian strain of *R. rattus* was obtained from T. Mbise, TPRI, Arusha, Tanzania.) The strains of both species are wild type and susceptible to anticoagulants. Although *R. rattus* was the main test species of the project, *R. norvegicus* was chosen for some preliminary tests as this species is rather particular about what it eats and, furthermore, in the first part of the project only a limited number of *R. rattus*, primarily males, were available.

Fleas

The strain of the rat flea *Xenopsylla cheopis* used in this study originates from Sokoine University of Agriculture, Morogoro, Tanzania. By modifying the rearing technique for rearing the squirrel flea, *Ceratophyllus sciurorum sciurorum* (Schrank, 1803), (LARSEN, 1994), we were able to rear *X. cheopis* on Danish rodent species. Two species of mice,

long-tailed field mouse *Apodemus sylvaticus* (L.,1758) and yellow-necked field mouse *Apodemus flavicollis* (Melchior,1834), were used for this purpose.

Newly emerged fleas and the mouse were put into the nest box (Fig. 1). One table spoon of dried bovine blood and one table spoon of sand were placed in the filter paper tray. The blood was added to ensure adequate food for the flea larvae. Sand was used because both larvae and adult *X. cheopis* fleas like to "hide" in sand (Frank Clark, *pers. comm.*). The environmental conditions in the room housing the flea cultures were 21°C and 80% RH. A photo period of 19:5 hour light:dark was used. This encouraged the mice to spend more time in the nest box with the fleas, because both species of mice used are most active during night (GELMROTH, 1969, 1970).

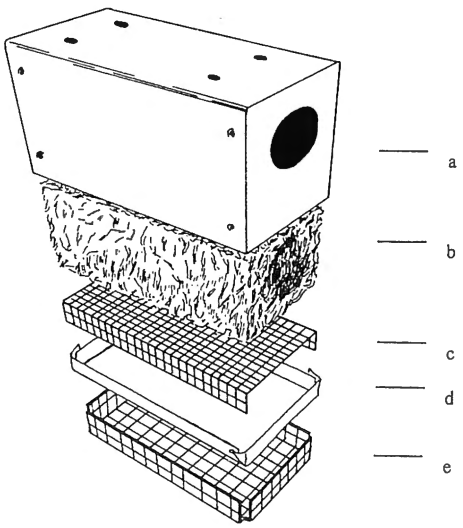

Fig. 1. – Mouse nest box.
a = stainless steel box,
b = straw material,
c = nest box floor (metal grid),
d = filter paper tray and
e = metal grid protecting tray.

When fleas were needed for supplementation of the cultures, flea eggs and larvae were obtained by sieving the nest box material plus the material of the filter paper tray in a sieving tower of a vibratory shaker (Retsch Laboratory Sieving Machine (VIBRO); Retsch, Germany). The eggs and larvae were collected from the sieved material and placed in tissue culture flasks with filter caps (Nunclon Flasks, Nunc, Denmark) containing larval food. The flasks were placed at 23°C and 80% RH.

When the adults emerged from the cocoons in the flask, they were transferred to a mouse nest. It has been experienced that sieving the nest material every second week will keep down the number of mites nourishing on flea eggs. The cultures produced about 300 adult fleas per week per mouse when the adult flea population in the mouse nest was kept on approximately 50 in number.

Palatability tests

The palatability tests were carried out as choice tests in a special test room at the DPIL (Fig. 2). This room was about 13 m^2 and equipped with nest boxes, water ad lib, and during

the test period a group of 12-20 trays in the centre of the floor. Before the start of a test, a group of 5 or 10 rats were introduced into the room and allowed to acclimatise for a period of at least three days. In this period the rats were offered normal laboratory food (Altromin pellets) on the floor; the trays in the centre of the floor were empty and placed as shown in Fig. 2. When the acclimatisation period was over, the non-poisonous Altromin pellets were removed and the rats were given the choice between the mixtures A and B alternating in the central trays. Mixture A was a bait base with 0.005% bromadiolone mixed with the insecticide in question or in some tests with *R. rattus* without insecticide. Mixture B was either 0.005% bromadiolone in the actual bait base (in tests with *R. norvegicus*) or the bait base alone as a non-poisonous placebo to mixture A (in tests with *R. rattus*). Tests with bromadiolone in both mixtures should give an indication of the effect on palatability of adding an insecticide, whereas tests with a non-poisonous alternative should simulate a field situation. The bait base used for *R. norvegicus* was the so-called EPA bait base (containing 65% ground yellow corn, 25% steamed rolled oats, 5% powdered sugar and 5% corn oil [MARSH, 1977]) whereas for *R. rattus* the bait base was crushed wheat.

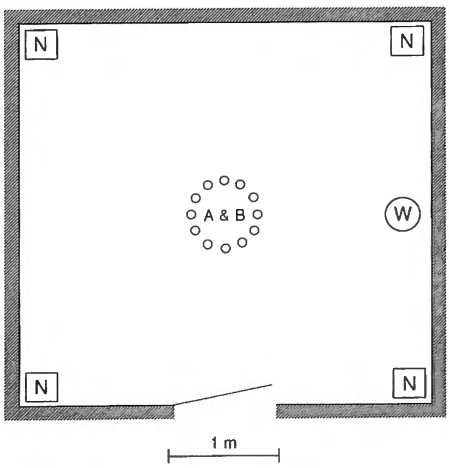

Fig. 2. – Ground plan of rodent test room. N=nest box, W=water, A and B=trays for the two mixtures alternating in a circle.

The palatability was tested over a four-day period. The four-day period was found suitable because death caused by bromadiolone often occurs from the fourth day in laboratory tests. The amounts consumed were recorded each day and the trays were refilled and the position of A and B interchanged. When the actual palatability test period was finished, it was followed by a three-week observation period for registration of any possible symptoms of poisoning and days to death. During this period, the rats were given Altromin pellets and/or rye bread. Dead rats were removed successively.

The tests with the DPIL *R. norvegicus* strain and with the Tanzanian *R. rattus* strain included both males and females whereas tests with the DPIL *R. rattus* strain were on males only.

The palatability of the baits was measured from the bait consumption and the mortality of the rats. The percentage bait acceptance (P) was computed by use of the formula $P = 100T/(T + S)$, where T is the weight of the treated bait consumed and S is the weight of the untreated bait consumed (THOMPSON *et al.*, 1972).

Flea toxicity test

The effectiveness of an insecticide given in a bait was tested on *R. rattus* infested with the rat flea *X. cheopis*. The DPIL strain of *R. rattus* was used. The rats were taken from the breeding colony and placed singly in glass terrariums. The terrarium was equipped with a metal nest box with nest material, a food tray and a water bottle. Each rat had an acclimatisation period in the terrarium of at least three days during which the food was non-poisonous organic crushed wheat. The nest material was renewed with fresh nest material at the end of the acclimatisation period and then followed a) a two-day period with daily recordings of consumed amounts of non-poisonous crushed wheat, b) a one-day starvation period, and c) a 4-, 5- or 6-day period with the combined bait preparation of the rodenticide and the insecticide to 3 or 4 rats in each test series (non-poisonous crushed wheat was given to a control rat). After the first day, each rat should have consumed at least 1 g of bait per 100 g body weight to be allowed to continue in the test. If consumption was satisfactory, 100 newly emerged unfed fleas were introduced into the nest box to each of the rats. The consumption of poisonous or non-poisonous bait respectively was recorded daily. As no rats were killed in the test due to the rodenticide intake, the rats were killed by CO_2 and frozen before examination for occurrence of fleas. The effect of the systemic insecticide was evaluated by collecting and counting the fleas alive in the nest material and from the rats themselves. Fleas found on the rats that had been frozen were recorded as live fleas.

Only unfed fleas newly emerged from the cocoon (less than 24 hours before the test) were used. This is important because a level of mortality (dehydration) can often be observed for fleas kept away from a host (especially those who already have been feeding on a host). Used in efficacy evaluations like the present, such weakened fleas would give an artificially high mortality as they are more susceptible to insecticides (own observation and EL-GAZZAR *et al.*, 1988) and are probably removed more easily by the grooming activities of their host.

RESULTS

The preliminary palatability tests where *R. norvegicus* was used (Table 1) showed that the least accepted bait was the one containing 0.24% phoxim followed by baits containing 0.3% and 0.15% fenthion, respectively. The commercially available microencapsulated fenthion product (Baytex ME 35%), which was tested in a concentration of 0.15%, was the best accepted insecticide formulation in the baits. In all tests using *R. norvegicus*, no alternative non-poisonous food was offered to the rats and a 100% mortality was observed.

TABLE 1

Bait acceptance and mortality for Rattus norvegicus *(DPIL strain, 5 males and 5 females) for baits containing different insecticides and 0.005% bromadiolone (BR). Alternative bait with 0.005% bromadiolone present.*

Bait	*Number of animals*	*Bait acceptance %*		*Mortality %*	
		Mean	*Range*	*Mean*	*Range*
Phoxim 0.24% + BR	2 x 10	5.2	3.2 - 7.2	100	-
Fenthion 0.15% + BR	2 x 10	16.4	11.8 - 20.9	100	-
Fenthion (ME) 0.15% + BR	2 x 10	23.3	10.8 - 35.8	100	-
Fenthion 0.3% + BR	1 x 10	7	-	100	-

Besides phoxim and fenthion, several kinds of dimethoate formulations were included in the tests with *R. rattus* (Table 2). The combined preparation of phoxim and bromadiolone gave a rather low intake clearly illustrated by the fact that the poisonous intake did not exceed 3.7% of the total consumption. No rats died in the phoxim experiments. The bait mixtures containing 0.15% fenthion were better accepted by *R. rattus* than those with 0.24% phoxim. The mean total intake over four days was 67.2 mg fenthion/kg body weight (b.w.). The use of 0.6% dimethoate in the bait mixture showed that it was possible to raise the bait acceptance level in general and the rat mortality level to 80%. The mean daily bait intake gave a dimethoate intake of 49.3 mg a.i./kg b.w.

Based on the observation in the palatability tests with *R. norvegicus* that the bait acceptance could be increased by using an encapsulated insecticide, encapsulated phoxim and fenthion were tested along with two types of encapsulated dimethoate (Table 2).

TABLE 2

Bait acceptance and mortality for Rattus rattus *(DPIL strain, males) for baits containing different insecticides and formulations and/or 0.005% bromadiolone (BR). Alternative non-poisonous bait present*

Bait	*Number of animals*	*Bait acceptance %*		*Mortality %*	
		Mean	*Range*	*Mean*	*Range*
Phoxim 0.24% + BR	1 x 5	4.6	-	0	-
Phoxim (C) 0.24% + BR	2 x 5	2.5	1.2 - 3.7	0	-
Fenthion 0.15% + BR	1 x 5	13.4	-	40	-
Fenthion (C) 0.15% + BR	2 x 5	20.7	7.2 - 34.2	60	20 - 100
Dimethoate 0.6% + BR	1 x 5	24.4	-	80	-
Dimethoate (X) 0.6% + BR	3 x 5	34.4	19.0 - 48.3	86.7	60 - 100
Dimethoate (Y) 0.6% + BR	2 x 5	19.5	17.6 - 21.3	70	60 - 80
BR	4 x 5	29.6	6.5 - 53.9	75	20 - 100

The test results showed that for only one of the dimethoate formulations (marked X) it was possible to raise the mean bait acceptance above what was found for the non-encapsulated formulation as well as above the level found for the bait containing bromadiolone only. The increased bait acceptance was also reflected in a higher rat mortality. This dimethoate formulation was chosen for further palatability testing on the Tanzanian *R. rattus* strain.

The test of baits containing the encapsulated dimethoate on the Tanzanian strain of *R. rattus* gave a remarkable difference in the bait acceptance between males and females, viz. 12.1% and 3.9% respectively (Table 3). The observed mean mortality was 95% for males and only 25% for females. There was thus a much lower bait acceptance for *R. rattus* males of the Tanzanian strain than for the tested males of the DPIL strain but a higher mortality rate for a lower total intake of rodenticide. The mean total intake of rodenticide over the four-day test period was 3.3 (DPIL strain) and 2.3 (Tanzanian strain) (in mg bromadiolone/kg b.w.). This indicates that the Tanzanian strain of *R. rattus* could be a bit more susceptible to the rodenticide than the DPIL strain.

TABLE 3

Bait acceptance for Rattus rattus *(Tanzanian strain) for baits containing insecticide and 0.005% bromadiolone (BR). Alternative non-poisonous bait present. M = males and F = females.*

Bait	*Number of animals*	*Bait acceptance %*		*Mortality %*	
		Mean	*Range*	*Mean*	*Range*
Dimethoate (X) 0.6% + BR	2 x 10 (M)	12.1	9.8 - 14.4	95	90 - 100
Dimethoate (X) 0.6% + BR	1 x 5,1 x 10 (F)	3.9	2.4 - 5.3	25	0 - 50

The 0.6% dimethoate formulation X was chosen for the flea toxicity tests. The intake of this bait formulation by the rats raised the mortality of the fleas compared to the rats given untreated bait (Table 4).

TABLE 4

Number of fleas found alive on the rats and in the nest material (N = number of rats)

Group	*N*	*Mean*	*Std Dev*	*Minimum*	*Maximum*
Treated	13	25.5	16.9	1	50
Untreated	4	59.5	9.2	53	73

DISCUSSION

According to CLARK & COLE (1971), who studied the effect of phoxim on *X. cheopis*, a single dose of 100 mg a.i./kg b.w. was the lowest effective dose given to guinea pigs. In the present tests, such a high value was not obtained as the mean total amount of phoxim

eaten over four days by the rats was about 15.6 mg phoxim/kg b.w. In a later study in outdoor pens (CLARK & COLE, 1974) a 0.24% phoxim bait in bait stations was found to give 99.3% reduction in flea index seven days after treatment. MILLER *et al.* (1975, 1977a, 1977b) obtained effective control of different flea species on native rodents in field tests also with 0.24% phoxim in the bait. An obvious reduction in the flea burden of the rodents was observed as early as 36 hours after treatment. In spite of these relatively positive results, our phoxim palatability experiments gave results that did not encourage further studies on the use of phoxim as the intake of this bait formulation was low and no rats died.

CLARK & COLE (1968) found that a mean daily intake of 4.1 mg fenthion/kg b. w. was the lowest dosage giving 100% kill of *X. cheopis* on hooded white rats. Thus, if the effect of the rodenticide had been higher than the 60% mortality we observed, fenthion would have been a good candidate for use *e.g.* in field trails.

In field tests with 0.24% and 0.36% dimethoate, MILLER *et al.* (1978) obtained a significant flea reduction on *Dipodomys* spp. and *Sigmodon hispidus* Say & Ord (1825). However, CLARK & COLE (1968) did not find dimethoate sufficiently effective against *X. cheopis* because the bait was not readily consumed. The daily intake was in their experiments over a fortnight up to 12.8 mg a.i./kg b.w. with hooded white rats. The lowest daily intake observed in our study was 33 mg a.i./kg b.w. so the 0.6% dimethoate in the X-formulation was chosen for the flea toxicity test.

The difference found in the flea mortality for the rats given the bait containing 0.6% dimethoate (X-formulation) plus 0.005% bromadiolone compared to those given untreated bait (74.5% and 40.5%, respectively) should be regarded as the minimum difference. In the former some of the fleas collected from the body of the rats as well as in the nest had probably taken a bloodmeal recently, having thus consumed the dimethoate. To be able to evaluate how this affects the fleas, the rats should not have been frozen at the end of the test period. The fleas collected then plus those collected from the rat nest material should have been observed for further 24 hours and the effect of any consumed dimethoate could then be demonstrated. Another question to be raised in this test is whether the fleas in the treated group of rats survive because the fleas had not taken a bloodmeal during the test period or just have not got a deadly dosage of the dimethoate from the blood of the host. Further testing is needed to clarify this. The relatively high mortality observed for the fleas in the control group, is probably due to the grooming activities of the rats.

The reduction in the flea population observed in the flea toxicity tests indicates that with the present formulation of 0.6% dimethoate as the systemic insecticide, it is possible to reduce the number of fleas on rodents along with the use of a rodenticide. However, as seen from the palatability tests, the amount of consumed insecticide/rodenticide bait drops when alternative food is available. Whether this intake of insecticide and rodenticide will be high enough to obtain a successful control of the rodent as well as the flea population in the field has to be tested. In the future we plan to continue using bromadiolone as the rodenticide, because it is an efficient slow-acting rodenticide. Concerning the choice of insecticide, many new types of insecticides have been developed in the last few years and some of these may have a potential for increasing the bait palatability and/or the flea mortality.

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EFFECTS OF NEEM PLANT (*AZADIRACHTA INDICA* JUSS, MELIACEAE) PRODUCTS ON MAIZE GRAIN CONSUMPTION BY THREE COMMON RODENT PESTS IN KENYA

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Abstract. We investigated the effect of neem plant (*Azadirachta indica*) products on maize grain consumption and body weights of *Lemniscomys striatus, Mastomys natalensis* and *Arvicanthis niloticus* live trapped in a Kenyan sub-humid grassland. The rodents were fed on oven dried crushed maize grains (mean wt=0.04 ± 0.003 g each, n=300) either plain, adulterated with neem derivative or with powder from leaf or fruit. Consumption over a five-day period and body weights (pre- and post-treatment) were recorded. Leaf derivative lowered consumption by 50.3%, 51% and 59.8% and the powder by 13.4%, 12.4% and 25.1%, respectively, by *L. striatus, M. natalensis* and *A. niloticus*. Neem fruit derivative and powder, respectively, depressed consumption for *L. striatus* (54.4% and 22.6%), *M. natalensis* (49.3% and 25.1%) and *A. niloticus* (60.4% and 27.7%). Post treatment body weights for all species were reduced by 9.3% (leaf) and 12.6%(fruit derivative) with a respective mortality rate of 7% and 20%. Our study showed that neem products significantly ($p<0.001$) lowered maize grain consumption in the three pests with the derivative being more effective than the powder ($p<0.05$). In the provision of effective repellent properties, formulation was more important than plant parts alone. *Azadirachta* products, due to their repellent effects, have potential in dry maize seed protection and may form a useful component in the development of an integrated pest management (IPM) strategy for rodents in Africa.

Key words: Neem, repellent, pests, *Mastomys, Lemniscomys, Arvicanthis.*

INTRODUCTION

One of the most serious problems facing developing countries is food security. In Africa, cereals are important staple foods. Although much is produced, large portions are lost through pest destruction or contamination at planting, growth, pre harvest, and storage. Rodents are known to attack crops at each of these stages. Some common rodent pests in sub-Saharan Africa include *Mastomys natalensis* (Smith, 1834) (a multimammate rat), *Arvicanthis niloticus* Desmarest, 1822 (the common grass rat) and *Lemniscomys striatus* L. (1758) (the striped grass mouse) (FIEDLER, 1994). In Kenya, these species cause major losses of field and stored grains (MARTIN *et al.*, 1989) but may also harbour deadly zoonotic diseases. Conventional control has involved trapping, digging, flooding burrows and, very often, the use of chemical poisons. Although rodenticide use can be expensive

(anticoagulants) or hazardous (acute poisons), they form an integral part of successful rodent pest management, and in some areas, the only practical method available (FIEDLER, 1994). This study assessed the repellant effects of *Azadirachta indica* (neem plant) derivatives on three sub-Saharan African rodents. The aim was to provide baseline information in the development of additional or alternative approaches to mitigating grain losses to rodents at planting and storage. Neem plant is widely available in Africa and easy to grow. It has known insecticidal properties (DUNKEL *et al.*, 1995) and limited evidence suggests that it may be useful as a repellent for birds (MASON & MATTHEW, 1996). In Kenya, it is widely used in the management of insect pests, and traditionally in the management of various protozoan, bacterial and fungal diseases. Neem tree products have been used for centuries in India for crop protection especially against storage pests (WATT, 1972). To our knowledge, evidence of its application for control of vertebrate pests in Africa is not documented and our study explores this approach.

MATERIAL AND METHODS

The study was carried out at Kenyatta University, 25 km north-east of Nairobi, Kenya (1°14′ S, 36° 48′ E). Rodents were collected from grasslands within the campus as described by OGUGE (1995). Captured animals were dusted (2-3 g) in insecticide powder (pyrethrin, 0.2%; piperony butoxide, 1.0%; inert ingredients, 98.8% w/w) identified, sexed, reproductive condition noted and weighed before being housed individually in metal cages (30 x 24 x 18 cm). Each animal was provided with commercial mouse pellets and water *ad libitum.*

Neem plant formulations

Fresh ripe fruits and mature green leaves of *Azadirachta* were obtained from Kanamai at the Kenya coast (3° 55′ S, 39° 47′ E). These were dried on air under shade to a constant weight (six weeks). The different plant parts were milled separately to a fine powder using an electric grinder and the powder packed in polythene bags. Storage was at room temperature (25° C) for 24-96 h, in a dark well-ventilated room, before extraction. Extraction was in methanol using the Soxhlet method (see FABRY *et al.*, 1977). To coat maize with neem preparations, crushed, oven-dried grains (108° C, 24 h)were soaked (100 g/50 ml neem extracts) either in derivative or powder for 24 h and then dried in an oven at 32° C for 12 h.

Feeding experiment

Rodents were first provided with plain, oven-dried (108° C, 24 h) maize (mean wt=0.04±0.003 g each, n=300) and water *ad lib.* Daily uptakes were recorded for five consecutive days. Untreated grains were replaced with derivative-treated ones, then powder-coated and finally plain grains, each treatment regime running for five days. Three rodent species were included in the tests: *M. natalensis*, *L. striatus* and *A. niloticus*. To assess for possible effects of extraction solvent on feeding, five *Tatera robusta* (Cretsch-

mar, 1830) individuals were simultaneously and similarly fed on grains soaked in methanol overnight and oven dried as above. Daily consumption was recorded for 20 days. In this design, each individual acted as its own control at pre- and post-treatment periods. Thus, any changes in adulterated-feed uptake could be directly assessed against a background of untreated ones.

Statistical analysis

Split-plot, split-split and one-way ANOVA were used to test treatment effects, variations between animal species and effects of different plant parts and formulations (powder & derivative) on feed uptake. Significantly different means were separated using Tukey-HSD tests. Regression analysis was used to assess for any relationship between feed uptake and time post treatment.

RESULTS

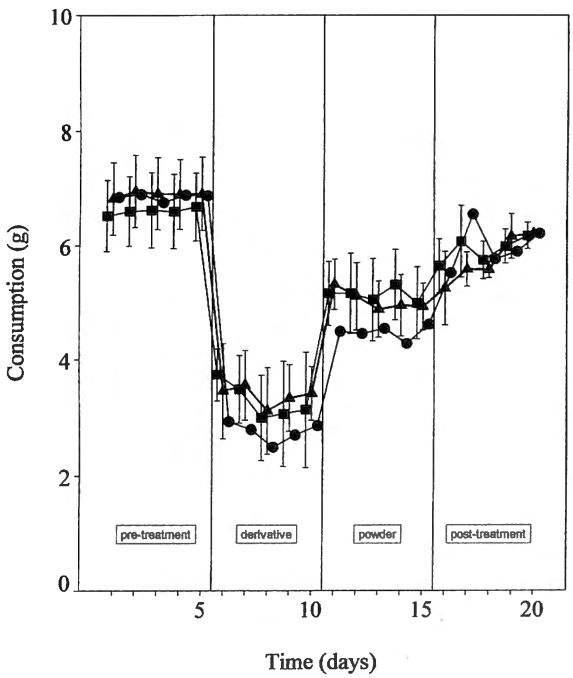

Fig. 1. – Mean (±SEM) consumption of crushed dry maize seeds by *Mastomys natalensis* (▲, n=5), *Lemniscomys striatus* (■, n=8), and *Arvicanthis niloticus* (●, n=2) over a 20-day period. The grains were either untreated (pre- and post-treatments) or adulterated with *Azadirachta indica* (neem plant) leaf products (derivative and powder). There was a highly significant ($p<0.001$) treatment effect. Powder-coated grains were more acceptable to the rodents than derivative-treated ones yet consumed in significantly lower quantity ($p<0.05$) than untreated maize seeds (post treatment) suggesting a repellent effect.

Following grain treatment with neem products, consumption by the three rodent species was significantly ($p<0.001$) reduced by an average of 52.3% (Figs 1-2). Leaf derivative reduced consumption in *A.niloticus* from a mean of 6.86 ± 0.05 g to 2.76 ± 0.15 g, a reduction of 59.8% (Fig. 1). *M.natalensis* consumption decreased from 6.9 ± 0.04 g to 3.38±0.15 g (51%) and in *L.striatus,* from 6.6±0.03 g to 3.29±0.28 g (50.3%). Consumption of grain, coated in leaf powder was also significantly ($p<0.05$) lower than the post treatment ones. These were by 25.1, 13.4 and 12.4%, respectively, for *A.niloticus, L.striatus* and *M.natalensis*. Similarly, maize adulterated with neem fruit products was consumed less by 60.4% (7.38±0.09 to 2.93±0.42 g) in *A.niloticus,* 54.4% (6.73±0.03 to 3.08±0.63 g) in *L.striatus* and in *M.natalensis* by 49.3% (6.62±0.05 to 3.35±0.46 g). Fruit powder also showed repellent effects as post treatment consumption of untreated grains increased by 27.7, 22.6 and 25.1%, respectively, for the three species. Variation in feeding by *T.robusta* throughout the 20 days was negligible (range=10.05±0.07 to 10.13±0.1 g).

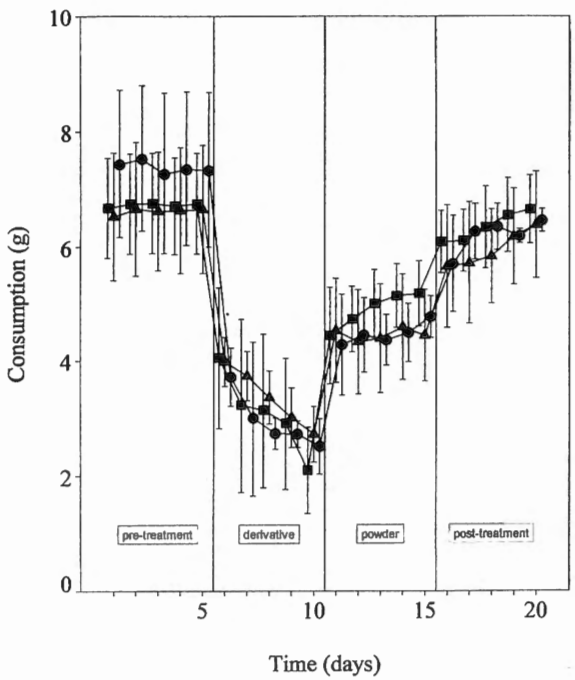

Fig. 2. – Mean (±SEM) consumption of crushed dry maize grains by *Mastomys natalensis* (▲, n=5), *Lemniscomys striatus* (■, n=11), and *Arvicanthis niloticus* (●, n=4) over a 20-day period. The grains were either untreated (pre- and post-treatments) or adulterated with *Azadirachta indica*'s (neem plant) fruit products (derivative and powder). Consumption was significantly ($p<0.001$) lowered by application of neem derivatives. As in leaf treatment, powder-coated grains were more acceptable to the rodents than derivative-treated ones yet consumed in significantly ($p<0.05$) lower quantity than unadulterated maize (post treatment) suggesting a repellent effect.

For the two plant parts, effects of derivatives were similar ($p>0.05$) but the effect of powder was different ($p=0.02$). Daily grain consumption following fruit derivative treatment reduced significantly ($R^2=0.77$, $p<0.001$, $n=15$) for all the species for the five-day period (Fig. 2). Although higher consumption was recorded during the subsequent powder treatments, post treatment consumption showed further increases with time always (Figs 1-2). Loss in body weight was observed in all individuals of the three species after the twenty-day period. The respective percentage weight loss for *M. natalensis, A. niloticus* and *L. striatus* following neem leaf treatment was 8.9, 9.7 and 9.3. Loss following fruit treatment was higher, *i.e.* 11.9, 11.2 and 14.6 respectively for *M. natalensis, A. niloticus* and *L. striatus*. A mortality rate of seven and 20% was recorded following leaf and fruit products treatments, respectively. The control animals registered a gradual increase in body weight and no mortality was recorded.

DISCUSSION

Our study has shown that *Azadirachta indica* products significantly ($p<0.001$) lower maize grain uptake by *Mastomys natalensis, Lemniscomys striatus* and *Arvicanthis niloticus* in a no choice experiment. *Azadirachta* contains the chemical azadirachtin (ASCHER, 1981), a product with unpleasant taste and smell. The concentrations of azadirachtin in crude extracts of neem trees in Kenya have been estimated at 1.5 and 7.2 mg/g for leaves and fruits, respectively (unpublished data, ICIPE, Nairobi). Although azadirachtin is the most important compound in seed kernels, others that provide less or synergistic effects are meliantriol, salannin, diacetylnimbin, nimbin, limonoids, vepaol, isovepaol and quercentin (SCHUMUTTARER & ZEBITZ, 1983). Neem plant concoctions may be consumed in fair quantities without apparent hazardous consequences (ARNASON *et al.*, 1989). Its seed kernel cake has been successfully used as protein-supplements for livestock (ANANDAN *et al.*, 1996; NAGALAKSHMI *et al.*, 1996; VERMA *et al.*, 1996). Thus, reduced uptake of neem-treated grains by the rodents here may have been due to a repellent rather than toxic effects. Similar findings have been shown (MASON & MATTHEW, 1996) in birds who avoid feed or water adulterated with neem preparations. In our study, consumption significantly decreased ($R^2=0.77$, $p<0.001$, $n=15$) over a five-day period in all individuals following neem fruit derivative treatment. In the few animals that died, consumption had reduced up to less than 0.5 g per day. The fact that surviving individuals increased their consumption post treatment (Figs 1-2) gives further credence to the suggested repellent effects.

Consumption in *A.niloticus* was affected most, being lowered by up to 60% compared with 54% in *L.striatus* or 50% in *M.natalensis*. These differences were highly significant ($p<0.001$). All individuals under treatment exhibited weight loss after 20 days. This may be attributed to lower quantity of grain eaten during that period. Since the individuals who died consumed very little, they may have starved. We found that the source of a neem product (the plant part from which it is extracted) was not as critical as the mode of preparation. For instance, fruit and leaf derivatives had similar depressing levels on consumption, while fruit powder was more effective ($p=0.02$) than leaf one. However, grain consumption was significantly ($p<0.05$) lower following derivative than powder coating. Therefore, in incorporating neem products in an integrated pest management programme,

the method of product preparation would be critical. Our study has shown that reducing maize grain consumption by *M.natalensis, L.striatus* and *A.niloticus* using *Azadirachta* concoctions is possible. Critical questions may arise in the use of this approach on grains used for human consumption. However, a recent study (DUNKEL *et al.*, 1995) has shown that stored beans (*Phaseolus vulgaris* L.) protected by ethanol extracted neem seed products were acceptable for consumption eight weeks after application. Acceptability of similarly protected maize grains is a possibility that needs exploring. Successful protection of mung beans and its crop, the chickpea (TIYAGI & ALAM, 1995) and of pigeon peas (AKHTER & MAHMOOD, 1996) by oil seed cakes of the neem against plant parasitic nematodes and soil-inhibiting fungi have been established. Use of this plant's products as repellents to rodents and other pests on seeds at planting may offer the seeds further protection and enhance germination (TIYAGI & ALAM, 1995). These preliminary findings from our study have shown strong repellent properties of neem plant products towards dry maize seed consumption by three species of sub-Saharan field rodents. We conclude that neem, used as a repellent, can provide an economical, biologically safe and socially acceptable approach that should be developed for integrated pest management for rodents in Africa.

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EFFECTIVENESS OF VARIOUS RODENT CONTROL MEASURES IN CEREAL CROPS AND PLANTATIONS IN INDIA

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Abstract. In India, crops vulnerable to rodent attack include rice and wheat cereals and coconut, cacao and oil palm plantations. *Bandicota bengalensis, Rattus rattus, Tatera indica, Meriones hurrianae, Millardia meltada* and *Mus* sp. are the principal rodents associated with crop damage. In rice 0.005% bromadiolone in bait stations at 15 m interval provided 88.3% control success. Based on live burrow count and baits placed directly in the burrows, the percent control was estimated at 98.8%. Increased yield was recorded in fields treated with bromadiolone as against those of warfarin, zinc phosphide and no treatment. Optimum time of control is six weeks after transplantation. In wheat, which is followed by rice in many areas, bromadiolone bait reduced more than 98% of the rodent population. In coconut plantations, with an average damage of 45.3 nuts/palm/year, amongst a single method of trapping only, warfarin baiting, zinc phosphide baiting and bromadiolone cakes, the latter provided 100% success at the rate of 2 cakes or 33 g/palm in treated plots as indicated by fallen nuts. Single climbing to the crown in case of bromadiolone is an extra advantage in the form of saving in labour in comparison to warfarin and zinc phosphide, also providing 100% success. Cacao intercropped with coconut were treated with bromadiolone wax cakes tied at the fonds at a rate of 2 cakes per tree. After 15 days of treatment, damage was completely reduced. The working index of Economic Threshold Level (ETL), schedule of control operations, monitoring techniques and other integrated measures are discussed.

Key words: *Bandicota bengalensis*, bromadiolone, damage, live burrow count, plantations, *Rattus*.

INTRODUCTION

India exhibits highly diversified habitats with extreme variation in climate. Such diversity favours a number of crops to grow and also harbours a broad spectrum of rodent fauna. There are 8 genera and at least 15 species, responsible for economic damage or public health nuisance in India (BARNETT & PRAKASH, 1975; PRAKASH & MATHUR, 1987, 1988). Rice is major cereal crop vulnerable to rodent attack. The losses may be as high as 3 to 100% in endemic zones, average being 4.9-19% (RAO & SINGH, 1983; REDDY, 1989, 1993). Wheat is damaged to the tune of 4-21% in Northern and Central India (ADVANI *et al.*, 1982; PRAKASH & MATHUR, 1988). Among plantations, coconut, cacao and oil palms are severely attacked by *Rattus* sp. and *Funambulus* sp. both in monocrop and intercrop.

For rodent pest management in cereal and plantation crops, chemical control is still an important component of the Integrated Pest Management (IPM), since it is the quickest and most effective method to decrease rodent depredation. However, the suitable timing of the operation, minimum effective dosage and proper follow ups are essential for an effective IPM module in these crops. The present communication reports on the effectiveness of bromadiolone (Roban) against rodents in rice, wheat, coconut, cacao and oil palm crops and other chemical and non-chemical measures which are usually considered in a decision making process for rodent control in these crops.

MATERIAL AND METHODS

Bromadiolone was used in its two formulations (formulated by Pest Control (India) Limited): 1) bromadiolone 0.25% powder concentrate – it was mixed with cracked cereals (1:49) and 2% vegetable (arachid) oil as an adhesive to prepare 0.005% bromadiolone treated bait and 2) bromadiolone 0.005% ready-to-use wax cake – the 100 g slab contains six equal sized cakes of approximately 16.6 g each.

Rice and wheat

Six one ha plots were selected for burrow baiting and three one ha plots were taken for pulsed baiting treatment in rice, with one plot of equal size as control in each case. However, in wheat, an area of 100 acre (40 ha) was selected for the treatment as well as control. Cracked rice and cracked wheat were used as bait carriers for treatment in rice and wheat crops respectively. About 15 g freshly prepared bait was wrapped in a paper and placed directly inside the live burrows. During the pulsed baiting in rice, 50 g of 0.005% bait was placed on day 1, 7 and 14 in 20 bait stations/ha so that one bait station was available at every 15 m on the bunds and dykes.

In rice, two census methods were employed viz. live burrow count (burrow baiting) and census baiting (pulsed baiting) where as the former was used in wheat treatment.

Live burrow count

All the burrow openings, occupied as well as abandoned, were plugged with mud in the experimental area. The reopened burrows were counted and baited the next morning. After completing the treatment and lag period (15 + 4 days), the burrows were again plugged late in the evening and counted the next morning (MATHUR & PRAKASH, 1984). The difference in the per centage of pre and post treatment was considered as reduction in rodent population.

Census baiting

Empty bait stations were placed in three plots, four days before the pre treatment census started for familiarization purposes. Ordinary bait was placed in the bait stations for three days before and after treatment and lag period. The amount of bait consumed was

measured to the nearest 0.1 g. Position of bait stations with rodenticide bait were altered slightly to remove the bias of pre-baiting (MATHUR & PRAKASH, 1984). The bait stations with complete takes of the bait material were replenished with double the quantity.

Coconut

Following treatments were carried out in coconut palms, with a control plot for each treatment about 250 metres away from treatment plots.

Two types of traps, snap traps and live traps, were used. A total of 100 palms were selected for evaluation. Fifty of the trees were selected for trapping at ground level and 50 for crown trapping. Further only 30% of the trees were trapped. The traps were prebaited for three days with vada, a fried gram (pulse) preparation; this was followed by trap setting on the fourth and fifth day.

Bromadiolone was evaluated in an area of 90 palms of which only 50% were baited with bromadiolone wax cakes at the rate of two to three cakes, placed at the base of the panicle, on either side of the crown. Warfarin was also placed as cake with 0.025% active ingredient. Out of the 100 trees selected, 47 were baited on day 1 and 5. Two to three 30 g cakes were placed at the base of the panicle. Zinc phosphide was tested in an area containing 107 palms, 50 of which were baited. After prebaiting for two days 2.5% poison baiting was carried out on the third day. The bait used was rice flour with 10% groundnut oil wt/wt. Bait (20 g) was packed into 10 cm x 5 cm polythene bags and placed on the crown.

Treatments were assessed by reduction in number of damaged and fallen nuts. The damaged nuts were counted for three days prior to each treatment and for two days post treatment and a lag period of 4 days. The reduction in mean nut fall before and after treatment were compared. The count on the first day of operation was discarded.

The cost:benefit ratios were calculated on the basis of cost of candidate treatment plus labour against cost in saving through reduction in damage.

Cacao

In a plot of 3 ha. with 295 trees and 155 coconut palms, one to two bromadiolone cakes were placed on 260 pod bearing cacao trees and two cakes on the crown of each coconut palm. Efficacy of treatment was determined by pre- and post-tratment counts of fallen/damaged pods.

RESULTS AND DISCUSSION

In rice fields, only *Bandicota bengalensis* (Gray, 1835) burrows were observed. After 10 days, the percent reduction as measured by decrease in number of live burrows was about 92% whereas activity reduction increased to 98.8% after 15 days (Table 1).

TABLE 1

Efficacy of 0.005% bromadiolone against Bandicota bengalensis *in rice. Census by live burrow count*

Plot	*No. of burrows treated*	*No. of reopened burrows after days*		*Per cent Control after days*		*Mean % Control*
		10	15	10	15	
1	45	02	00	95.6	100	
2	48	06	00	87.5	100	
3	54	03	02	94.5	96.3	98.8
4	60	06	01	90.0	98.4	
5	58	05	00	91.4	100	
6	57	03	01	94.8	98.2	
Control	50	48	49	04.0	02.0	

During pulsed baiting (Table 2), 0.68 to 0.78 kg bromadiolone bait was consumed per ha in the 3 pulses. In majority of the trials with bromadiolone in rice where *Bandicota bengalensis*, *Mus booduga* (Gray, 1837) *Millardia meltada* (Gray, 1837) are prevalent, the reduction in rodent population has been reported to be 80-100% (CHOPRA, 1988; REDDY, 1989; SIVAPRAKASAM & DURAIRAJ, 1992; MATHUR *et al.*, 1992; BASKARAN *et al.*, 1995). Lower percent control as computed by census baiting in comparison to live burrow count can be attributed to consumption of plain bait by non target animals like ants and birds etc. (MATHUR & PRAKASH, 1984). However, it can be inferred that burrow counting is more accurate and can be fairly applicable as a monitoring technique since bandicoots and other field rodents in India live a solitary life in their burrows (BARNETT & PRAKASH, 1975), except that females live with young ones till they are weaned.

In an earlier study (anonymous pers. comm.), observations on mean tiller damage and grain yield in the treatments revealed that when bromadiolone bait was applied on bunds and fields, grain yield was highest and tiller damage was lowest in comparison to treatments with zinc phosphide and warfarin.

It has been reported (CHOPRA, 1988; SIVAPRAKASAM & DURAIRAJ, 1992; REDDY, 1993) that rodent control in rice should be undertaken 30-40 days after transplantation beyond which the efficacy of chemical control decreases considerably because rodents shift their preference to rice panicles.

In the 100 acre area of wheat, 1128 burrows were located and treated at the rate of 15 g bromadiolone bait in a paper packet per burrow. On the 10th day, 65 live burrows were observed, showing a 94.3% population reduction. After 15 days, when bromadiolone produced its maximum effect, the number of live burrows was only 21 *i.e.* a reduction of 98.1% of the prevailing rodent population comprising of *B.bengalensis* (65%), *M.meltada* (24%) and *M.booduga* (11%). In the control plot 500 metres away in similar habitat pre-treatment live burrow count was 976 which after 15 days during post treatment census in treatment plot, marginally increased to 992. The percent rodent control in wheat with bromadiolone

in different agro-climatic zones in India including the present study (MATHUR *et al.*, 1992) indicate that bromadiolone provides a very high level of control in wheat crop. ADVANI *et al.* (1982) reported that with 85% control success, the increase in yield of wheat can be 249-368 kg/ha. In wheat, the damage is visible from very initial stages when seeds are sown. One control operation at this stage (November-December) and other one near maturity (February-March) can reduce the damage to a large extent (MALHI *et al.*, 1986).

TABLE 2

Efficacy of 0.005% bromadiolone pulsed baiting in rice as measured by census baiting

Plot	*Pre-treat/Post treat plain bait consumption (g) for 3 days*	*Bromadiolone bait consumed/ha (g)*	*% Success*	*Mean*
1	883/92	780	89.5	
2	785/95	720	87.9	88.3
3	668/83	685	87.5	
Control	725/738	-	-	

Rattus rattus (L.) and *R.r.wroughtoni* Hinton, 1919 are the most predominant species in coconut (SHAMSUDDIN & KOYA, 1985; ADVANI, 1986; BHAT & SUJATHA, 1991; present study). In the coconut nurseries, *B. bengalensis, Tatera indica* (Hardwicke, 1807), *M.booduga* and *M.meltada* were trapped. In the present study, rodent damage to nuts by *R.rattus* were observed to the tune of 45.3 nuts/palm/year or 51.1% at a density of 150 palms/ha. The damage caused is 3.72 nuts/palm/month amounting to Rs.16,995/year/ha (US $ 472).

In the 130 snap traps laid, only one *M. booduga* was trapped whereas nothing was trapped in wooden live traps placed both at ground and crown level. The decrease in damage to the nuts was insignificant where trapping treatment was given (Table 3). Bromadiolone, warfarin as well as zinc phosphide baiting at crown level resulted in 100% reduction of damaged, fallen nuts. RAO *et al.* (1984) found 100% and 84% reduction in rodent population consequent to bromadiolone and warfarin baiting respectively. SHAMSUDDIN & KOYA (1985) and BHAT & SUJATHA (1991) also obtained 100% control of *R.rattus* on coconut palms using bromadiolone. The cost of operation for bromadiolone, warfarin and zinc phosphide works out to Rs. 87.50, 90.50 and 112.95 respectively for almost equal number of palms. The higher cost of zinc phosphide baiting was due to labour charges since the palms had to be climbed for two days prebaiting and one day baiting.

In cacao and coconut intercrop, a single treatment resulted in 100% reduction in damage after 15 days of treatment. *Rattus r.wroughtoni* and *Funambulus tristriatus* (Waterhouse, 1837) were principal species in cacao. BHAT & SUJATHA (1991) obtained 100% kill of *R.r.wroughtoni* with bromadiolone yet for squirrels, the maximum kill achieved was only 50%. The higher level of control in the present study is attributed to baiting on both cacao and coconut trees so that squirrels had access to the bait even at nesting sites. Trapping with wooden or wire mesh live traps is recommended for 100% pro-

tection to cacao pods from squirrels (BHAT & MATHEW, 1983). Increase in harvest from 12 to 21 times in a year has been found to reduce squirrel damage from 52% to 25% (ABRAHAM *et al.*, 1979). In the present study, the cost of rodenticide for treating 415 palms was Rs.1155/- (US $ 32/-) for 10.5 kg bromadiolone cakes. In other words, cost of treatment per palm is Rs.2.78 i.e approximately 12 palms can be treated for a US dollar which is within affordable limits, looking into the magnitude of damage. Oil palm is another upcoming crop in India and 400,000 ha are being added to boost oil palm production. Considerable damage is caused to this crop by rodents, but studies indicated that bromadiolone provides good protection to oil palm at all stages (SUBIAH & MATHUR, 1992).

TABLE 3

Comparison of four methods of rodent management in coconut plantations

Treatment	*No. of palms*	*No. of fallen nuts/day*		*% damage prevented*	*Cost Benefit Ratio*
		Prior to treatment	*After treatment*		
I BROMADIOLONE					
1 Control plot	90	11.0	13	-	-
2 Treated plot	87	15.0	-	100	1:67
II WARFARIN					
1 Control plot	80	8	6	25.0	-
2 Treated plot	100	17	-	100	1:54
III ZINC PHOSPHIDE					
1 Control plot	90	6.33	4.9	22.6	-
2 Treated plot	107	14.33	-	100	1:50
IV TRAPPING					
1 Control plot	75	15.66	13.66	-	-
2 Snap traps	211	19.33	12	-	-
3 Live traps	435	51.66	40	-	-

With high densities and severe losses and low budgets dedicated by the cereal growing farmers to rodent control, long-term continuation of management strategy is not usually followed. It is therefore, necessary to evaluate the efficacy of each method and the optimum timing to get the maximum out of it. The threshold level of 15 burrows/ha is worked out on the basis of number of burrows usually observed at the time of the crop stage when the damage is picking up, hence, it becomes the optimum time to take up a chemical control measure, which can bring the damage curve down. Since most of the field rodents in cereal fields are burrow dwellers, live burrow count is an effective monitoring technique (MATHUR & PRAKASH, 1984). It is interesting but frustrating that rodents do not accept rodenticidal baits adequately after panicle initiation stage. Hence, it is more useful from that stage onwards to go for trapping, using local traps or fumigation by employing smoke

generators or aluminium phosphide tablets. The agronomic practices coupled with cultural control methods (bund trimming and weed control) also play crucial roles. In high return plantation crops, the options are few and second generation anticoagulant baiting has proven effective even as a single treatment (one exposure of rodenticide) in most of the situations. It can, however be integrated by keeping the crowns, ground and palm circles clean to reduce rodent harbourages. Live trapping and baiting on trees which are nesting sites for squirrels enhance the success of the operation. Rodenticides and trapping are still important components of IPM for rodents, however, the use of ecological parameters like density in relation to crop stage, damage appraisal, reinfestation pattern and proper timing of suitable control measures, strengthen the decision making process. The present study indicates that bromadiolone provides excellent rodent control success in cereals as well as in plantations by integrating proper formulations, placement and timing of control operation. Warfarin and zinc phosphide also provided 100% kill in coconut plantations but the lengthy baiting process with the former and necessity of prebaiting and a follow up programme with the latter weigh the balance in favour of second generation anticoagulants.

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IMMUNOCONTRACEPTION AS A POTENTIAL CONTROL METHOD OF WILD RODENT POPULATIONS

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Abstract. Rodents have the capacity to periodically reach very high numbers in agricultural landscapes, reducing agricultural production, and causing considerable environmental and social problems for farmers and their families. Such rodent problems occur worldwide and have a long history. Currently, mortality enhancing agents (mainly poisons) are the principal method of rodent control. This approach raises environmental, ethical and humane issues, and ignores the inherent high capacity for increase in these species. We argue that it is more appropriate to reduce reproduction than increase mortality. This paper uses house mice in Australia as a case study to explore fertility reduction as a potential alternative to conventional methods of control. In particular, the question of what level of fertility control is required to have a significant effect on population growth is discussed. A computer simulation, based on the life-history strategies of house mice, examined the effect of different levels of fertility control on mouse population dynamics. This simulation provides a reference for future studies of confined populations of mice used to test the effects of fertility control, refines the design of these experiments and identifies the type of data needed to be collected. Immunocontraception, the process of inducing the body's immune system to attack its own reproductive cells, is suggested as a method for reducing fertility in rodent populations. The advantages and disadvantages of immunocontraception over mortality-enhancing agents are discussed, as are the potential impacts of social structure on the efficacy of immunocontraception and the possible application of this control method to other rodent pest situations, particularly rodent pest problems in Africa.

Key words: Fertility reduction, rodents, immunocontraception, mouse plagues, *Mus domesticus*, computer simulation, experimental design.

INTRODUCTION

Rodents have been the scourge of human populations since before Aristotle, 350 BC (THOMPSON, 1910), and in recent times have caused considerable losses to a variety of growing crops and to stored grain in Asia (GEDDES, 1992; SINGLETON & PETCH, 1994), Africa (LEIRS *et al.*, 1997), Australia (SINGLETON & REDHEAD, 1989; CAUGHLEY *et al.*, 1994), and elsewhere (see PRAKASH, 1988; BUCKLE & SMITH, 1994). Rodents also play an important role as carriers of zoonoses such as plague, leptospirosis, hantaviruses, Lassa fever and leishmaniases (CHILDS *et al.*, 1994; GRATZ, 1994; references in this issue).

Another major concern, which has become prominent in recent years, is the impact of introduced rodents on the conservation of native wildlife, especially on islands (WACE, 1986; MOORS *et al.*, 1992; KEY *et al.*, 1994; COWAN & TYNDALE-BISCOE, 1997).

There are two principal stategies for managing rodent pest populations - increase mortality or decrease fertility. Currently, the main method for controlling rodents in agricultural landscapes relies on increasing mortality using poisons, particularly anticoagulants. There are problems associated with the use of chemicals for control. These have been discussed elsewhere (SINGLETON & REDHEAD, 1989) and are summarised as follows.

1. Residues can contaminate the growing crop, soil and any nearby water supplies.
2. Non-target deaths can occur due to primary poisoning by consumption of bait (granivorous species) and secondary poisoning from consumption of rodent carcasses (predatory or scavenging species) (see also SAUNDERS & COOPER, 1981 for examples).
3. Large areas need to be treated if re-invasion from neighbouring areas is to be minimised, making baiting an expensive option.
4. There are ethical and animal welfare issues with respect to the suffering inflicted on the animals during poisoning (see also SAINSBURY *et al.*, 1995; OOGJES 1997).

Predation is a natural mortality-enhancing factor in rodent population dynamics. The effectiveness of predators in regulating field populations of rodents has been demonstrated (*e.g.* KORPIMÄKI & NORRDAHL, 1989; SINCLAIR *et al.*, 1991; JAKSIC *et al.*, 1992). ANDERSSON & ERLINGE (1977) concluded that generalist and migrating specialist predators can stabilise rodent populations, particularly during and after the decline phase in the rodent population, but predation is not as successful in regulating populations which are increasing or already high.

Rodents display typical *r*-species attributes: they are small, highly mobile, fecund, short-lived, have a wide niche breadth, and a variable "boom and bust" population density (SOUTHWOOD, 1977). Therefore, control methods which concentrate on increasing the level of mortality need to be well targetted or highly effective if they are to be successful. Even then, the effect is generally short-lived given the life-history characteristics of rodents. A biological method of control, particularly one which reduces the high reproductive capacity of rodents, could be a more effective alternative.

The prospects for controlling rodents using biological methods have been reviewed by SINGLETON (1994) who concluded that it was preferable to use agents which reduced fertility rather than increasing mortality. This is viewed as an appropriate control approach for vertebrate pests generally (*e.g.* CAUGHLEY *et al.*, 1992).

The primary aim of this paper is to examine fertility reduction as a possible means of controlling rodents. An important question that needs to be addressed is what level of fertility control is necessary to produce a sustained effect in wild populations? A case study of house mice in Australia is used to examine this question. A computer simulation of two levels of sterility allowed us to examine the possible effects on population growth rate and abundance of mouse populations compared against an unsterilised control population. The simulation will be used to assist in the design of enclosure experiments in which wild mouse populations, housed under semi-natural conditions, will have a proportion of the females surgically sterilised to mimic the effects of a fertility-reducing agent.

The concept of immunocontraception as a possible way of achieving sterility is discussed and the advantages and disadvantages of this method compared with mortality enhancing methods currently available for controlling rodents. Immunocontraception is then discussed in terms of its likely effects on rodent social structure and its application to rodent pest situations other than house mice in Australia, particularly rodent pests in Africa.

CASE STUDY

The house mouse, *Mus domesticus* (Schwarz & Schwarz, 1943) causes considerable economic and social stress to rural communities due to its ability to form plagues at irregular intervals in the grain-growing regions of eastern and southern Australia (SINGLETON & REDHEAD, 1989; CAUGHLEY *et al.*, 1994). It would be unrealistic to expect that mice could be completely eliminated from these areas. Rather, the aim should be to decrease mouse populations so that the degree of damage inflicted is at or below the level that causes economic hardship to growers.

Results from studies of mouse populations in Australia's cereal-growing regions indicate that average litter size is greater in the 12-18 months prior to a plague than at other times (SINGLETON & REDHEAD, 1990). In the mallee wheatlands, the length of the breeding season is longest 12 months prior to a plague (SINGLETON, 1989). Previous modelling of mouse population dynamics suggests that if these occasional seasons of high mouse productivity were prevented, mouse plagues may not occur (REDHEAD, 1987).

HONE[1] produced an empirical estimate of the level of fertility control required for reduction in population growth from the intrinsic rate (r) to zero in 13 mammalian pests, including mice. The estimate for mice was determined indirectly using rates of increase and a generation interval obtained from field data (REDHEAD, 1982). HONE predicted that the proportion of females that needed to be sterilised was 0.60. Two points arise from this. One is that immigration would increase this estimate. The other, is that for much of the time the rates of increase of populations of mice are likely to be less than r and it is probably not necessary to reduce the rate of population increase to zero, depending on the timeframe and damage thresholds.

Computer simulation

To simulate the effects of sterility, we constructed a simple demographic model of a mouse population with three age classes (juvenile, 0-5 weeks; sub-adult, 5-6 weeks; and adult, >6 weeks) and three reproductive classes (male, intact female and sterilised female). Only sub-adults and adults were in the trappable population (SINGLETON, 1987). The assumptions and parameters used in this model are summarised in Tables 1 and 2.

[1]HONE, J. (199-) – How much fertility control of vertebrate pests is enough? *J. Anim. Ecol.* (submitted).

Software to implement the model was written in-house in the Pascal programming language.

TABLE 1

Assumptions underlying demographic modelling of the predicted effect of surgical sterilisation on enclosed, wild mouse populations

Assumption	*Justification*
Breeding synchronous within and across enclosures	Based on previous experiments using wild mice in the enclosures (see BARKER *et al.*, 1991) and the Whitten effect (WHITTEN, 1966).
Survivorship equal across sexes and age cohorts	Based on BARKER *et al.* (1991)
No compensation in birth or death rates with density or treatment	Compensation detectable by comparing actual data to predictions from model
Sex ratio of progeny is 1:1	
All females bred that were capable of breeding	Resources in excess

TABLE 2

Life history and population parameters included in demographic modelling of the predicted effect of surgical sterilisation on enclosed, wild mouse populations. a.b. = after birth

Parameter	*Estimate*	*Reference / Justification*
Litter size	5.25 (SD = 1)	From wild mouse colonies used to derive founder mice
➥ Gestation period	➥ 19 days	
➥ Juvenile period (includes weaning period	➥ In nest 21 days; on surface 14 days (0 - 5 weeks a.b.)	Wild mouse colonies and
➥ Age at sexual maturity	➥ 42 days (6 weeks a.b.)	WHITTINGHAM & WOOD (1983)
Sub-adult period (trappable but not sexually mature)	7 days (5 - 6 weeks a.b.)	SINGLETON, 1987
Minimum period between litters	Includes lactational diapause 23 days	WHITTINGHAM & WOOD (1983)
Survivorship	97% per 7 days (99.5% per day)	Based on BARKER *et al.* (1991)

Each simulation began on day zero with 12 adult females and 8 adult males in each population and ran for 140 days with a daily time step. A founding population of 20 was chosen as this was likely to be the initial population per replicate in future studies of confined populations of mice used to test the effects of fertility control. There were three treatments: all founder females intact, 67% of founder females sterilised, and 75% of founder females sterilised.

Because the initial number of mice was small, we used integer arithmetic to represent the number of mice in each state. A daily mortality rate of 0.005 was assumed for all age-sex classes and binomial samples were removed daily from each class to simulate natural mortality. We assumed that intact females had ready access to males and so became pregnant on day one of the experiment and gave birth 19 days later. To allow for the fact that a small number of female mice have a lactational diapause which delays the onset of parturition (WHITTINGHAM & WOOD, 1983), the gestation length was set to 23 days rather than 19 days, as shown in Table 2.

To sustain the level of sterility imposed on each treatment population, the females from the first litter produced by the founders ($F1_1$ litter) were sterilised at the same level as the founder females.

As it is likely that the experimental enclosures will provide mice with ample space for nest sites and unlimited access to food (assuming social influences are not important for inhibiting access), the model assumed no density dependence in mortality rates and that all intact females produced litters. Litter size was taken as a normally distributed random variable with mean 5.25 and standard deviation 1.0 rounded to the nearest integer (see Table 2 for justification of this litter size). A binomial sample from each litter was assigned to each sex class (male/female) such that the expected sex ratio was 1:1.

Because the model is stochastic, the mean of ten runs was used to predict trappable population during the course of the experiment (Fig. 1). This was done for populations with 0%, 67% and 75% sterility of females. The control population (0% sterility) was higher than the sterilised populations and there was a difference between the 67% and 75% level of sterility.

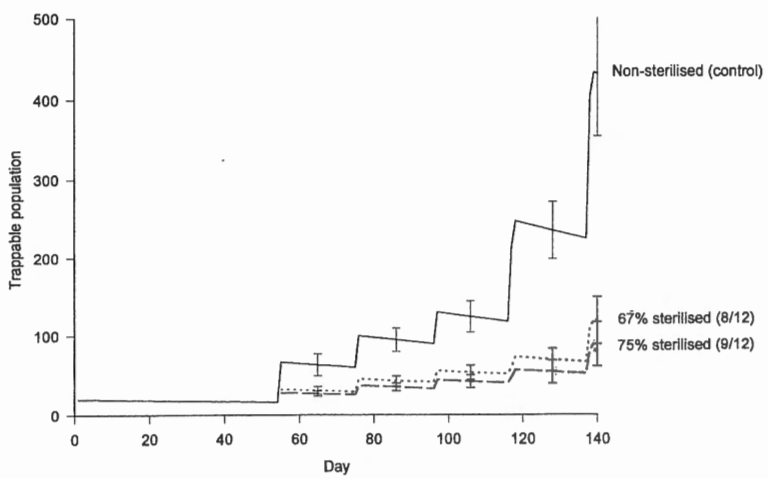

Fig. 1. – Predicted trappable population of wild mice housed in outdoor enclosures where a proportion of the females (0%, 67% and 75%) have been sterilised. Each plot is the mean of 10 runs of a demographic model with the variance shown (±S.D.) at each step of the plot. $F1_1$ to $F1_4$ indicates when F1 generation litters (those produced by the founding population of mice) will enter the trappable population (5 weeks of age – see Table 2). $F2_1$ indicates when the first litter of the F2 generation (produced by the first F1 generation litter – $F1_1$) enters the trappable population.

Each step in the plots indicates the entry of a new cohort of mice into the trappable population. The first three litters ($F1_1$ to $F1_3$) are litters produced from the founding population and are the first generation. The fourth F1 litter becomes trappable at the same time as the first litter of the second generation ($F2_1$). The experiment would need to cease at or just after day 120 when the $F2_1$ litter enters the trappable population and before the females from this group produce a litter. This means that all mice recruited into the trappable population have come from cohorts with the appropriate level of sterility.

Regular sampling times during the experiment can be located using Fig. 1. It would be best to sample the population just after each new cohort has entered the trappable population – around days 60, 80, 100, and 120. This will allow changes to population size to be monitored as they occur.

We plan to test the results from the simulation by conducting studies of confined populations of wild house mice surgically sterilised to mimic a fertility reducing agent. Deviations from the model may allow the importance of compensation in treated populations to be determined. The effects of compensation were not included in our simulation but they have been found to be important in similar experiments examining fertility control for rabbits (WILLIAMS & TWIGG, 1996). The simulation has already assisted in determining the types of data that need to be collected (Table 3) and will assist further by refining the design of these enclosure experiments (*e.g.* how often and when should population sampling occur).

TABLE 3

Parameters to be measured for enclosure populations of wild mice surgically sterilised to simulate immunocontraception (based on modelling exercise)

Parameter	*Justification*
Population size (trappable)	To compare with output from model
Proportion of females breeding	Tests the assumption of synchronous breeding and success of fertile females breeding (*i.e.* pregnant and/or lactating)
Survivorship of adults and trappable juveniles	Compare with level imposed in model
Litter size at autopsy at the completion of the experiment	Compare with values used in model
Recruitment	Measure of survival of neonates

DISCUSSION

The simulation of house mouse abundance over time, under the proposed conditions of an enclosure experiment using wild mice, indicated that by sterilising two-thirds of the females in the population, it was possible to have a substantial effect on population abundance and growth rate. The simulation was used also to refine the design of an enclosure experiment to examine the effect of sterility on the dynamics of wild, semi-natural mouse populations. From the graph of the predicted trappable populations over time for the various sterility scenarios (Fig. 1), key sampling points were identified, as was a suitable termination point.

One method for reducing fertility in wild pest populations such as rodents, is the relatively new concept of immunocontraception. This method has attracted much attention internationally (TYNDALE-BISCOE, 1997) and research is in progress on the use of immunocontraception for the control of mouse populations in Australia (SHELLAM, 1994).

Immunocontraception

Immunocontraception is the process of inducing the body's immune system to attack its own reproductive cells (TYNDALE-BISCOE, 1994). The feasibility of this method for controlling feral mammals in general was first discussed in 1987 and presented at a conference on fertility control in wildlife held in 1990 (TYNDALE-BISCOE, 1991). It was identified that a biological control method which reduces fecundity, and is environmentally benign and humane, would satisfy many of the ethical, environmental and ecological criteria now required of a control method for vertebrates (BOMFORD, 1990).

The proposed approach for the mouse is to use a mouse-specific virus, murine cytomegalovirus (MCMV), as a carrier for a fertility-associated protein which will induce the body's immune system to block fertilisation. This approach is termed viral-vectored immunocontraception (VVIC) and has been identified as the most promising long-term control strategy for house mice in Australia (SINGLETON, 1994).

The fertility-associated protein used to promote an immune response could be from the egg, the sperm or other parts of the reproductive tract. At present, the best prospect appears to be a peptide from the mouse zona pellucida gene, ZP-3, which has been shown to cause long-lasting contraception in mice (MILLAR *et al.*, 1989).

Field studies examining both the distribution (SMITH *et al.*, 1993) and the prevalence of MCMV (SINGLETON *et al.*, 1993), and laboratory studies of the virus itself and its infection characteristics (see SHELLAM, 1994 for review), suggest that MCMV has excellent credentials to be a carrier of an immunocontraceptive for mice. One of this virus's most important features is that, being a cytomegalovirus, it is likely to be species-specific. The virus is widespread in wild mouse populations and has a high seroprevalence (>90%). Up to four strains have been isolated from individual mice and laboratory studies show that infection of laboratory mice with multiple strains can be achieved (BOOTH *et al.*, 1993). This is important if we hope to infect wild mice with a recombinant strain of MCMV in the presence of field strains of the virus.

A disseminating virus rather than a non-disseminating agent is preferred for delivery of an immunocontraceptive antigen because:

1. It has the potential to induce stronger immune responses and greater immunological memory.
2. It has the potential to spread a contraceptive protein rapidly through a mouse population.
3. It is much cheaper than using baits as a delivery agent because it can be «released and forgotten».
4. A species-specific carrier ensures that only the target species is affected.
5. The dynamics of a naturally disseminating virus is more likely to match the short generation time of mice.

The advantages of VVIC compared to methods of control which increase mortality are summarised in Table 4. VVIC has a more appropriate demographic target (fecundity rather than mortality); is species-specific by nature of the reproductive protein, carrier virus and method of transmission (ideally sexually transmitted); and because the vector is a self-disseminating virus, allows large areas to be treated at low cost.

There are potential risks associated with the use of VVIC. These are summarised in Table 4 and discussed in detail in TYNDALE-BISCOE (1994, 1995) and WILLIAMS (1997). Almost all of them relate to the issue of species-specificity and public acceptability. Hence there will need to be rigorous testing of related (and some non-related species) before any VVIC agent is released into the environment. However, it is important to view these risks in the context of the environmental and social acceptability of current management methods, and to weigh the benefits from these methods against those provided by VVIC.

TABLE 4

Advantages of viral-vectored immunocontraception (VVIC) for mouse control in Australia compared with agents which enhance mortality, and the risks and concerns associated with VVIC

Advantages of VVIC over current mortality agents of rodents	*Risks and concerns of VVIC*
Targets reproduction	Irretrievable once released
Self-disseminating, «release and forget» strategy	Public acceptance of genetically engineered organism being released
Species specific	Risk of recombinant virus losing species-specificity with time
Humane	Virus may infect laboratory colonies of mice
Large areas can be treated at minimal cost	International concerns re sterilisation of «desirable» *Mus spp.* (*e.g.* native to that country)
Environmentally benign	

Social structure

The importance of maintenance of social status amongst reproductive females in populations subjected to some level of sterilisation needs to be addressed (COWAN & TYNDALE-BISCOE,1997). Moreover, CAUGHLEY *et al.* (1992) identified types of social organisation and mating systems of target species that will not respond to immunocontraception. These findings have important consequences for the type of reproductive protein used in a VVIC agent (*i.e.* contraceptive versus castrative) and whether or not dominant females are targetted for fertility control.

Although it is difficult to examine the social structure of wild populations of mice, enclosure studies (*e.g.* CROWCROFT, 1966; SINGLETON & HAY, 1983) suggest that reproductive females have a social hierarchy which may determine whether or not a female will acquire mates. In female mice, the mainenance of a high social status is likely to be hormonally controlled. If so, an immunocontraceptive which leaves immunised females hormonally intact, allowing them to maintain their social position in the population and continue to suppress reproduction in subordinate females, would be preferred.

If dominant females contribute disproportionately to the number of offspring produced in a population, then the contraceptive agent should target these females but not compromise their social status. The breeding performance of socially subordinate females which remain fertile needs to be maintained at a low level, otherwise these animals may compensate for the reduction in population growth (COWAN & TYNDALE-BISCOE, 1997).

In rodents, the relevance of social status to fertility control through immunocontraception could be examined experimentally by comparing populations where females have been surgically sterilised using ovariectomy and tubal ligation. Ovariectomy results in females being hormonally compromised, possibly disrupting the social ranking of the female. Tubal ligation leaves the hormonal system intact, retaining any social order that is hormonally controlled.

Immunocontraception and its potential application to other rodent pests

The use of this approach in other rodent pests and the type of vector used will depend on the status of the target. There are three possible approaches for two types of targets:

(1) For totally undesirable, exotic species, with no closely related native species present, then a naturally disseminating virus could be used as the vector.

(2) For native species that reach undesirably high population densities in specific areas, then it would be preferable to use a bait containing the immunocontraceptive protein for strategic, localised control. In this situation, it also may be necessary to have the option to reverse the effects if required (TYNDALE-BISCOE, 1991). The short generation time for rodents may necessitate frequent baiting, depending on the persistence of infertility and the level of bait uptake. An immunocontraceptive baiting program could therefore be costly.

(3) An alternative approach to (2) for native pest species is the use of a fertility agent which is not persistent but is disseminated widely by a viral vector. If an animal develops immunity after infection and then subsequently recovers fertility, then the effect is to dampen the population peaks.

The success of any of the above will depend on the ability of the population to compensate through increased survival or breeding performance. Rodent species with a high intrinsic rate of increase, r, are likely to compensate for the level of immunocontraception but will be less likely to undergo large fluctuations in population density (see SINCLAIR, 1997). In Africa, many of the rodent pest species typically have large fluctuations in population density and generally only cause economic problems in agricultural systems when they are at high densities (see LEIRS *et al.*, 1997). Fertility control via immunocontraception therefore could be an effective, humane and environmentally benign method for rodent management in Africa.

In contrast to the situation with house mice in Australia, most of the rodent pests in Africa are not exotic species - their numbers need to be managed in agricultural and urban situations but the goal is not to eliminate them. Moreover, the average area occupied by each farm is at least two orders of magnitude smaller than that of Australian grain-growers. The logistics and costs associated with distributing a sterility bait over a couple of

thousand hectares and on a number of occasions during the breeding season of the rodent pest, are less tractable than for families in Africa who farm 0.5 to 5 hectares.

For these reasons, the baiting option (option 2) is likely to be more appropriate to the African situation. The success of a baiting program will depend on the life-history characterisitics of each species (their ability to compensate via improved survival, high emigration or increased breeding performance of those which remain fertile) as well as the cost to conduct multiple baiting. If VVIC is socially acceptable and is the only economically viable option of those available for immunocontraception, then option 3 may be appropriate. However, the use of VVIC is a long term option which requires much research effort and a detailed and thorough process of public consultation and of close scrutiny by regulatory authorities (see WILLIAMS, 1997).

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INTEGRATED MANAGEMENT OF RODENTS: A SOUTHEAST ASIAN AND AUSTRALIAN PERSPECTIVE

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Abstract. This paper discusses the concept of integrated pest management (IPM) and considers the progress that has been made towards effective implementation of IPM for rodent pests in agricultural systems in Southeast Asia and Australia. Unfortunately, progress with the management of rodents lags considerably behind IPM for insect pests and diseases of crops. Too often, recommended management practices lack scientific rigour, instead they are based on frequent reiteration of a concept which results in it being accepted as dogma. From a rodent management perspective, IPM in these regions is better described as perceived integrated management (PIM). Two case studies, one from Southeast Asia and one from Australia, are presented to demonstrate how replicated, manipulative field experiments with appropriate controls can redress this situation. The first study is on the rice field rat in West Java. The second study is on mouse plagues in southeastern Australia. In each case, the IPM programs are built around detailed descriptive studies of the population ecology of the pest species. The challenge lies ahead for rodent wildlife managers to not only develop effective rodent IPM but also to integrate these management actions with existing IPM programs of non- mammalian pests. From the perspective of a wildlife biologist, other pressing challenges for establishing effective and sustained control of rodents in Southeast Asia, were identified. These were the lack of appropriate tertiary training in wildlife management, the weak infra-structure for research on rodent pests, and the need to develop effective extension for programs on the management of rodents.

Key Words: Integrated pest management, rodent, mouse plagues, population ecology, Southeast Asia, Australia, *Rattus argentiventer*, *Mus domesticus*.

INTRODUCTION

Integrated pest management (IPM) is simply defined as the integration of a range of management practices which together provide more effective management of a pest species than if they are used separately. IPM has been the principal goal of the development of pest management for many years, although the chief goal of an IPM program is often not clear to practitioners involved in that program. A recent survey indicated that staff in the United States of America who coordinated the extension of a federal IPM program on insect pests were diametrically opposed to whether the chief goal of their IPM program was to reduce pesticide use; 20 thought yes and 23 thought no (GRAY, 1995). This highlights the need for managers of rodent IPM programs to learn from the mistakes of the past.

IPM had its genesis in the control of insect pests and plant diseases in agricultural environments where there was a strong move away from pesticides towards management of the agricultural system so as to promote methods which were least ecologically disruptive (SMITH & VAN DEN BOSCH, 1967). These methods include physical, cultural and biological control. A common approach for managing insect pests and diseases of crops is to promote the effect of predators and competitors through adopting actions which have a minimum impact on their natural role of limiting pest populations. These predators or competitors can be either endemic to a region or introduced as part of a biological control program aimed at establishing an alien organism in the region. At this point I wish to emphasise that rodenticides and biological control agents (if they can be successfully applied) should both be considered as integral parts of IPM of rodents. Too often views are expressed that a particular program is not IPM because poisons were used or their use was not reduced (GRAY, 1995), or once biological control is developed no further action will be required. Both views are incorrect and are old arguments. In the first instance, the use of rodenticides is consistent with IPM principles if the amount and/or frequency of applications of the rodenticide have been significantly reduced during the implementation of IPM (see BUCKLE, 1990; FIEDLER & FALL, 1994, for discussion). Although there is an equally acceptable view that IPM is not designed to reduce the use of chemicals for controlling pests, rather the goal is to reduce the impact of the pest with minimal impact on the environment (PETTY, 1973).

In the second instance, the coevolution of the biological disease agent and the host (the pest species) is likely to reduce the effectiveness of the biological control. Therefore, maintenance of effective control will require the action of the biological agent to be augmented by more traditional methods of management. This is the clear message emerging from one of the few successful cases of biological control of a vertebrate pest; the use of a myxoma virus to control rabbits in Australia (WILLIAMS *et al.*, 1995). Finally, the need to consider the use of either chemical or biological control in a broader ecological context is paramount given the toxicological effects of inappropriate use of chemicals and cases of biological control which not only have been ineffective but have caused worse ecological problems (*e.g.* the release of the cane toad in Australia and Hawaii).

There are many socio-political factors which influence the adoption rate of IPM (see KENMORE *et al.*, 1985; NORTON & HEONG, 1988 ; POSAMENTIER, 1988). The use of decision analysis (NORTON, 1982; NORTON & PECH, 1988) early in an IPM program helps to address these factors through understanding why farmers adopt particular management actions and ignore others. A decision analysis approach provides the platform for developing and extending management actions through matching science to the problem. I will not dwell on the socio-political influences on IPM, instead I will narrow the focus to the scientific approach to integrated management of rodents. Too often recommended management practices lack scientific rigour, instead they are based on frequent reiteration of a concept which results in it being accepted as dogma. Unfortunately, wildlife management in general suffers from the acceptance of hypotheses that are not supported by rigorous field data. SINCLAIR (1991) provides an elegant discussion of science and the practice of wildlife management.

Development of a regional or national IPM program requires not only rigorous scientific research but also implementation of basic management principles. From a research

perspective, to focus simply on good scientific method is folly if the scale, setting (laboratory versus field studies) of experiments, and/or the management actions being examined are inappropriate (see also NORTON & PECH, 1988). A decision analysis approach helps marry research goals with management goals. From a management perspective, the strength of rodent IPM is dependent on the following principles:

(i) The management actions are environmentally sound – the use of rodenticides are minimised or targeted so as to reduce their impact on non-target species and, depending on the chemical, their entry into the food web (especially as a residue in the crop being protected).

(ii) The management actions are cost effective – this is of particular importance in developing countries where farmers have small holdings and little disposable income for management actions.

(iii) The management actions must be sustainable – this relates not only to actions being environmentally sustainable, but also to actions being consistent with the other demands on the end users. If the actions are too complex, too labour intensive or too difficult to integrate with their farm management system, then the end users are unlikely to either sustain their actions or maintain the requisite quality of actions.

(iv) The management actions must be able to be applied on a large scale – this is particularly important given the mobility of rodents and their ability to quickly colonise areas where crops are ripening and where rodent numbers have been reduced by local control actions.

(v) The management actions are politically advantageous – an action may be both feasible and desirable but is not adopted for local or national political reasons (see NORTON, 1988 for example).

In this paper the scientific approach to the integrated management of rodents will be demonstrated by drawing on two case studies of research currently being conducted in Southeast Asia and Australia. Both studies are aimed at critically evaluating IPM programs using replicated and controlled field studies.

CASE STUDIES

Case study 1: Rice field rat in Indonesia

The Rice field rat, *Rattus argentiventer* (Robinson & Kloss; 1916), is the most important pre-harvest pest in Indonesia (GEDDES, 1992). Rat damage to rice crops is the greatest agricultural problem in Indonesia causing annual production losses of approximately 17% (see SINGLETON & PETCH, 1994 for review), equivalent to reducing the potential value of the Indonesian rice crop by about US$ 1 billion.

The principal methods of rat control in Indonesia are varied, generally widespread and labour intensive. However, there have been few efforts to critically evaluate the effectiveness of these methods, either singly or in combination (Table 1).

An emerging and promising method of control is the use of a trap-barrier (TBS) to protect rice crops, which was first developed in Malaysia (LAM, 1988). The TBS consists of

a rectangular or linear fence and multiple-capture traps inserted at intervals near the base of the fence. A mud mound, which protrudes above the irrigated water level, provides access to the traps. It works on the principle that rats enter the edge of the flood-irrigated crop, but cannot gain access to the rice because of the fence. Rats could climb over the fence but rarely do so. They swim along the fence, taking the line of least resistance, come to a mud mound and then enter the trap. Up to 129 rats have been caught in a single trap (260 x 260 x 620 mm) in one night (LAM *et al.*, 1990).

TABLE 1

An overview of what is known about the most common methods used by farmers for controlling the rice field rat in Indonesia

Method of rat control	*Level of adoption by farmers*	*Research on effectiveness*	*Potential as part of IPM*
Poisoning	Moderate; prefer zinc phosphide – cheap & see rat bodies	Yes – but best data on anticoagulants	High – depends on benefit-cost and availability
Trapping	High-labour intensive	None	Medium
Rat drives	Moderate – labour intensive but only when rat numbers are high	None	Low? – timing important
Electrocution	Low – high elsewhere in SE Asia	None	Low
Natural predators	Low – generally few birds of prey; snakes are hunted	None	Medium
Fumigation	High – labour intensive; using a sulphur gas	None	Medium – timing important
Trap-barrier	Moderate – usually with no traps; labour intensive	Yes, but no controls	High – depends on benefit-cost

The principle behind the TBS is simple and is now widely used in many countries in Asia. However, benefit-cost analysis indicates that rat damage would need to be higher than 30% in rice crops for the method to be cost effective (SINGLETON *et al.*, 1994; LAM YUET MING, pers. comm.). There have been various claims that the effectiveness of the TBS can be enhanced if the crop inside the TBS («trap-crop») is at an earlier stage, later stage, more aromatic, consists of seedlings (rice is transplanted into adjacent rows every two weeks) or is a combination of these treatments. In some instances these claims were backed up with data, but in each case there were inadequate control sites, usually no replication and limited economic assessment. Instead of critical evaluation of well formulated

hypotheses, poorly substantiated claims were feeding off each other which led to the development of the dogma that «trap-crops» inside a TBS will provide general protection from rats for surrounding rice-crops. This dogma may indeed be correct but the data available were insufficient to provide farmers with a prescriptive approach to the type of trap crop, the size of the crop (and TBS) and how frequently they need to be spaced.

To redress this situation a project has been established in West Java, Indonesia, with replicated treatment and control sites, and rigorous assessment of the benefit-cost to farmers. From an IPM perspective, this study has been assessing also the effectiveness of fumigation of rat burrows using sulfur gas. Fumigation is the most common method of rat control used by farmers in this region.

Design of TBS and Fumigation study

The study is located at the Research Institute for Rice in Sukamandi, West Java. There are four treatments with two replicates per treatment. The treatments are a TBS with fumigation of rat burrows; TBS with no fumigation; fumigation only; no rat control. Each site is separated by a minimum of 500 m. The rice within each TBS (variety IR64) is transplanted 14-21 days prior to the surrounding crop (variety IR64). At sites without a TBS, a similar area is planted with rice 14-21 days prior to the surrounding crop.

Each TBS consists of a 50 m x 50 m square of 0.7 m high plastic supported by bamboo poles (1.2 m long) inserted 0.5 m into the ground, spaced 1 m apart and interconnected by string. The bottom 50-100 mm of the fence is buried. A live-capture trap is placed every 25 m (n=8), flush with and opening to, the outside of the fence. These are multiple capture traps (600 mm x 240 mm x 240 mm) with an opening of 100 mm in diameter at the base of one end of the trap leading to a wire cone 240 mm long, tapering to 50 mm in diameter. The traps are made of open wire mesh (gauge 1 mm and 12 x 12 mm squares). Holes are made in the fence to allow entry into the traps. There is raised earth above the water level at each entry point.

Rat burrows along the perimeter of each 5 ha site were fumigated using sulphur gas. The gas was delivered by a hand-operated fumigator which forced air over smouldering straw containing sulphur granules. Fumigation was conducted every 1 -2 weeks after the rice crop was at maximum tillering stage.

Damage caused by rats to rice tillers were assessed along 6 transects to the north and 6 to the south of the trap crop, every two weeks and for each treatment. The transects were within the trap crop, and 5 , 50, 100, 150 and 200 m from the trap crop. Each transect was 11.2 m long, following a transplanted row of rice. Every fifth hill (n=10) along each transect was assessed for number of tillers damaged by rats. In addition to cut tillers, yields were assessed from 10 x 10 m quadrats taken at each transect and at each of the eight sites during the week prior to harvest.

An overview of the outcomes from the first two years of the study is presented in Table 2. Briefly, the TBS provided good returns to growers when rat numbers were high relative to other seasons and most of the rat damage was during the generative stage of the rice crop (booting to harvest). Fumigation had little effect on rat damage to rice crops, resulting in a net cost to growers using fumigation for rat control. In one of three seasons,

the TBS plus fumigation treatment resulted in better control of rat damage, and hence yield loss, than the use of a TBS alone.

TABLE 2

An overview of the effectiveness of a trap-barrier system (TBS), fumigation or TBS plus fumigation for controlling the rice field rat in West Java, Indonesia. The benefit-cost ratio was estimated from differences in yields between treated and untreated fields of rice. Where two values are given these are a direct and a more conservative estimate (see SINGLETON et al., in press for details).

Season	*Rat density*	*Time of main tiller damage*	*Benefit-cost ratio*		
			TBS	*Fumigation*	*TBS + Fum*
1995 Dry	High	Booting to harvest	20: 1 to 7: 1	Net cost	Additive effect
95/96 Wet	Low	Maximum tillering	7: 1 to 2: 1	Net cost	No effect
1996 Dry	Medium	Tillering	Net cost	Net cost	No effect

These results highlight the need for longitudinal studies of management practices with concurrent data on the population dynamics and ecology of rat populations, before a robust IPM program for rats can be developed for a particular region.

Case study 2: Mouse plagues in Australia

In southern and eastern Australia, populations of house mice, *Mus domesticus* (Schwarz & Schwarz, 1943), occasionally erupt with densities of >1,000 ha^{-1} occurring over thousands of square kilometres. These mouse plagues cause substantial economic losses, high levels of social stress, health risks to humans, and environmental problems through the heavy use of chemicals (see SINGLETON & REDHEAD, 1989 for review).

The occurrence of mouse plagues is aperiodic. On average there is 3 to 7 years between plagues depending on the region. It is difficult therefore to develop and critically assess management actions within a short time frame. Indeed, it is only recently that we have been able to obtain sufficient ecological data to determine key processes leading to the formation of mouse plagues (SINGLETON, 1989; BOONSTRA & REDHEAD, 1994; TWIGG & KAY, 1995) or to focus on key issues which must be addressed (KREBS *et al.*, 1995). Currently there is pressure from farmers and society to formulate IPM rather than continue with the current practice of remedial large scale use of poisons once a plague has developed.

In southeastern Australia, scientists and farmers have collaborated to identify what are likely to be the best practices available for managing mouse populations given our current knowledge. A demonstration study was established in two regions as part of a national program for establishing best practice for managing vertebrate pests (see BRAYSHER, 1993).

The respective contributions by scientists and end-users (farmers) to this program are summarised in Table 3. Meeting with farmers at the beginning of the study provided consensus on issues such as:

TABLE 3

Integrated management of mouse plagues in southern Australia – the marriage of science and end-users

Contribution by scientists	*Contributions by farmers*
1. Habitat use and population dynamics of mice – what, when & where to control; scale of operation.	1. Identify actions to target factors (identified by science) which limit mouse populations – how to control.
2. Monitor population numbers & breeding of mice – interpretation and forecasts.	2. Logistics of control operations. – practicality & cost – possible to incorporate in farm program – consistent with sustainable farming?
3. Rigorous evaluation of management actions, including benefit-cost.	3. Important role in extension – facilitate adoption by neighbouring farmers.

(i) recognition that an integrated management approach is required,

(ii) that we have a solid understanding of the dynamics and habitat use of mouse populations and, although there are many gaps in our scientific knowledge, we can formulate testable strategies for managing mouse populations,

(iii) that rodent management procedures will be adopted only if they do not compete with existing farm management practices.

Design of study on Best Practices for Management of Mouse Plagues

The study began in June 1995 and is being conducted in two regions of western Victoria, Australia. In the first region there are four farmers conducting identified best

TABLE 4

Actions to be taken in spring by growers who are trialing recommended best practices for managing mouse populations in southern Australia. Lists of activities are available for the other seasons

Recommended spring activities
1. Control the growth of grasses and weeds along fences – spray before their seeds are set (seeds of early grasses may trigger breeding by mice)
2. Graze pasture well to minimise seed set of grasses
3. Reduce ground cover for mice around silos and farm buildings
4. Mouse-proof grain and stock food storages
5. Bait farm buildings and key habitats (margins of crops) in late September and October
6. Monitor signs of mouse activity in different habitats; bait where activity is high

practice for managing mouse populations (experimental regime) and three farmers doing what they have been doing for decades (control regime). In the second region there are two farmers in each regime. The farms have been matched as far as practicable for soil type, climate and crops. All have only one growing season per year (winter-spring), with the principal crops being wheat, barley, field peas and canola (oilseed). The study area on each of the 11 farms is between 800 and 1,000 ha and there is a minimum of 5 km between farms.

A range of actions have been identified for each season. As an example, the actions for spring are listed in Table 4. The actions by individual farmers are monitored and these are contrasted with changes in mouse abundance (live-trapping every 6 to 8 weeks), plant biomass along fence lines (measured in spring and autumn), grain remaining in fields immediately after harvest and 3 months later, and the level of mouse damage to crops just prior to harvest and at planting. Costs on implementing management practices are being collated to enable a benefit-cost analysis of the study.

DISCUSSION

IPM or PIM?

What progress have we made with rodent IPM in Southeast Asia and Australia?

In Southeast Asia, there have been detailed studies of aspects of the biology of the rice field rat, *R. argentiventer*, and the Philippine rice field rat, *Rattus mindanensis* (Taylor, 1934), in and around rice crops, and the Malaysian wood rat, *Rattus tiomanicus* (Miller, 1900), in oil palm plantations, which have led to recommended IPM programs (FALL, 1977; WOOD & LIAU, 1984; RICHARDS & BUCKLE, 1986; BUCKLE, 1988; 1990, COLVIN, 1990). Apart from replicated and controlled studies of the efficacy of various rodenticides for managing rat populations (*e.g.* BUCKLE *et al.*, 1984; LAM, 1990) there have been few studies which have evaluated critically, under field conditions, the effect of individual management actions let alone whether two different actions complement each other, are synergistic, are not additive or possibly have a lower effect than either action by itself.

In Australia, there have been detailed studies of aspects of the biology of house mice in cereal growing regions and of the canefield rat, *Rattus sordidus* (Gould, 1858), in and around sugar plantations. For the mouse there have been manipulative, replicated field studies of the effect of parasites (*e.g.* SINGLETON *et al.*, 1995; SINGLETON & CHAMBERS, 1996), food quality (*e.g.* BOMFORD & REDHEAD, 1987) and rodenticides (*e.g.* MUTZE, 1993; BROWN *et al.*, 1997) on mouse populations. Apart from the case study described above, no studies have evaluated critically the interaction and consequences of two different management actions for controlling mouse populations.

For the canefield rat, there has been a replicated manipulative study of different tillage practices (WHISSON, 1996). This work has been taken a step further through examining the efficacy of tillage practices in combination with restricted and targeted rodenticide usage for managing canefield rat populations (J. WILSON, pers. comm.).

Therefore, from a rodent management perspective, IPM in Southeast Asia is better described as Perceived Integrated Management (PIM). The situation in Australia is little better.

The Way Forward - Manipulative Experimental Field Studies

The adoption of successful IPM for rodents requires rodent biologists to establish first descriptive population studies. Ideally, these would be capture-mark-release-recapture studies conducted every 3-6 weeks, with traps set in the principal habitats in the cropping landscape. Such studies of rodent pest species are an essential precursor for establishing which strategic management approaches would be the best candidates for IPM. If there are sufficient data (minimum of 3 years data for chronic pest species; longer periods for outbreaking species) then modelling of the population dynamics of rats may enable critical evaluation of key population processes, the dynamics of habitat use, and/or an ability to forecast population outbreaks. Unfortunately, the modelling approach has been seldom used because there are few long term population studies of rodent pests.

Once the best prospects for IPM have been identified, hypotheses need to be developed and then critically evaluated using replicated, manipulative field experiments with appropriate controls. The control (untreated) sites in these manipulative studies provide continuity of previous population studies and hence the basis of a long term data set. Typically, few studies move beyond the descriptive population phase.

Rodent wildlife biologists therefore need to establish a good baseline ecological study, from there develop best prospects for IPM, then critically evaluate these management methods using replicated manipulative experimental studies in the field.

This is the basic philosophy behind the IPM case studies presented for the rice field rat and house mice. The case study for the TBS is not an isolated study. As part of a multinational program on rodent pest management in Southeast Asia, the TBS approach is being evaluated critically also in peninsular Malaysia and in the Mekong and Red River Deltas in Vietnam. This is an exciting development because this collaboration provides an opportunity to contrast the effectiveness of a rodent management program under different landscapes, agricultural systems, climates and socio-political backgrounds.

CONCLUDING REMARKS

Much has been written about the implementation of IPM for insect pests and plant diseases (see KILGORE & DOUTT, 1967; REISSIG *et al.*, 1986). In Southeast Asia, the entomologists and plant pathologists have joined forces to develop multicountry IPM programs. Rodent IPM lags substantially behind and thus far there has been only cursory consideration of rodents in these programs (VAN ELSEN & VAN DE FLIERT, 1990). The challenge lies ahead for rodent wildlife managers to not only develop effective rodent IPM but also to integrate these management actions with existing IPM programs of non-mammalian pests.

From the perspective of a wildlife biologist, there are other pressing challenges for establishing effective and sustained control of rodents in Southeast Asia. First, the exper-

tise in rodent biology needs to be strengthened; there are few tertiary courses in wildlife management in the region, with most practising rodent biologists having been trained as entomologists. This is of concern because the field techniques and analytical methodologies are markedly different for insects and rodents, as are their population dynamics (dealing with over-lapping generations with rodents), individual and social behaviour (more complex for rodents), and their population responses to climate, land management, diseases and predators. Second, the infra-structure for research on rodent pests is weak in most countries in the region. Third, the few research programs on rodent pests in the region focus primarily on single species. Generally, there are many rodent species living in or near rice fields. We need to be cognisant of the life history parameters of these species so that we can anticipate and monitor the possible emergence of one of these as a significant pest, if the population of the current primary pest species is managed effectively . Finally, effective extension of IPM programs has been difficult with insect pests (NORTON & HEONG, 1988). These difficulties are likely to be compounded for rodents because they have lived commensally with humans for centuries and there are many myths, taboos, rituals and customs linked with them. These vary from region to region, country to country. Once an IPM program has been shown to be effective, scientifically, in every likelihood there will be an even greater challenge to develop effective extension and adoption of the program. Recognition and then definition of these possible problems is an important first step and underlines a commonality between the scientific and sociological aspects of wildlife management – involve the end-users at the planning stage of studies, not after spending many years seeking and then defining what might be an inappropriate management program.

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COMMUNICATION IN NATIONAL RODENT MANAGEMENT PROGRAMMES

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Abstract. Despite of various national and international activities regarding the management of rodent pest populations, many recent publications still bemoan the fact that technical recommendations and control programmes are not implemented or sustained. The author is of the opinion that most programmes emphasise the aspect of technical solutions and overlook the importance of communicating the appropriate information to the relevant people at the right time. Therefore, for programmes to be successful, they should include a comprehensive communication component, which goes beyond merely passing technical information to farmers. This paper discusses and describes the need to motivate farmers to implement technical solutions; why and how to include intermediaries, who carry messages and physical inputs to the farmers, into a communication plan; and the role of decision makers, who are instrumental in legitimising local activities necessary for the success of a programme.

Based on the experience in several countries, the author argues for the need of including a communication component into a rodent management programme and describes some relevant aspects necessary in designing such a component.

Key words: rodents, control, managment programmes, communication channels, communication messages, extension, training, pre-test.

INTRODUCTION

Despite considerable research (BUCKLE & SMITH, 1994; PRAKASH, 1988) into rodent control techniques and many rodent control programmes around the world, present and past, rodents still pose a recurring problem in agricultural systems of many countries (QUICK, 1990; FIEDLER & FALL, 1994). Invariably surveys show that rodents are considered a major or even the number one pest, but recommendations are not implemented despite this awareness. The reasons may be a lack of political will, recommendations are too complex for those who should apply them or a lack of good technical solutions (FIEDLER & FALL, 1994).

Most rodent control programmes are managed by rodent specialists. These specialists often overlook the needs of farmers because they are too concerned with technical solutions. However, there are signs that this problem is recognised: a meeting of rodent specialists at IRRI, Philippines (QUICK, 1990) identified the need for further research into control techniques incorporating the principles of integrated pest management including

biological control. However this meeting also pointed out that extension staff and other intermediaries have to be trained and equipped adequately, so that farmers can be informed about and implement rodent control strategies.

This paper discusses the benefits of including a well designed communication component into rodent management programmes. Regarding the design of programmes in developing countries, it emphasises the importance of an analysis of farmer needs and the identification of groups directly or indirectly involved in rodent control and their potential impact on programme implementation. The discussion is based on the experience of the author, from which some solutions are drawn and suggested.

SITUATION ANALYSIS

The first step in programme planning is performing a situation analysis, collating current knowledge on the pest species and the crop of interest, determining how farmers can readily access relevant knowledge and materials for implementing effective management and examining the strengths and weaknesses of the existing infra-structure necessary for the programme (see also ALBRECHT *et al.*, 1989). The needs, expectations and problems of the target group are assessed through «knowledge, attitude and practice» (KAP) surveys, designed specifically to provide information for planning the programme and providing benchmark information for evaluation purposes (ADHIKARYA, 1994).

From this situation analysis, farmer opinion and observation surveys, general observations and interviewing people familiar with the local situation, we prepare a problem analysis. These problems are sorted into cause-effect relationship producing a «problem tree», with the effects at the top and the respective causes below (Fig. 1). It shows the relationships between problems, identifies information gaps and forms the basis for the programme plan. Figure 1 is such a tree, coalescing information from interviews conducted in Bangladesh, Myanmar and the Sudan. It will form the basis of the discussion to follow.

We may decide from this tree that the key problem is «rodents cause excessive damage» and therefore the goal of the programme is «rodent damage is reduced efficiently». If the government is involved in rodent control farmers consider the government to be responsible for their fields (Table 1). In the case of Sudan with large numbers of sharecroppers 32% of the farmers considered the owner to be responsible, who in general did not participate in the costs of inputs.

TABLE 1

Percentage of farmers considering the government responsible for rodent control in their fields (adapted from ADHIKARYA & POSAMENTIER, 1987; POSAMENTIER, 1991 and unpubl. data)

Bangladesh	*Myanmar*	*Sudan*
58	95	27

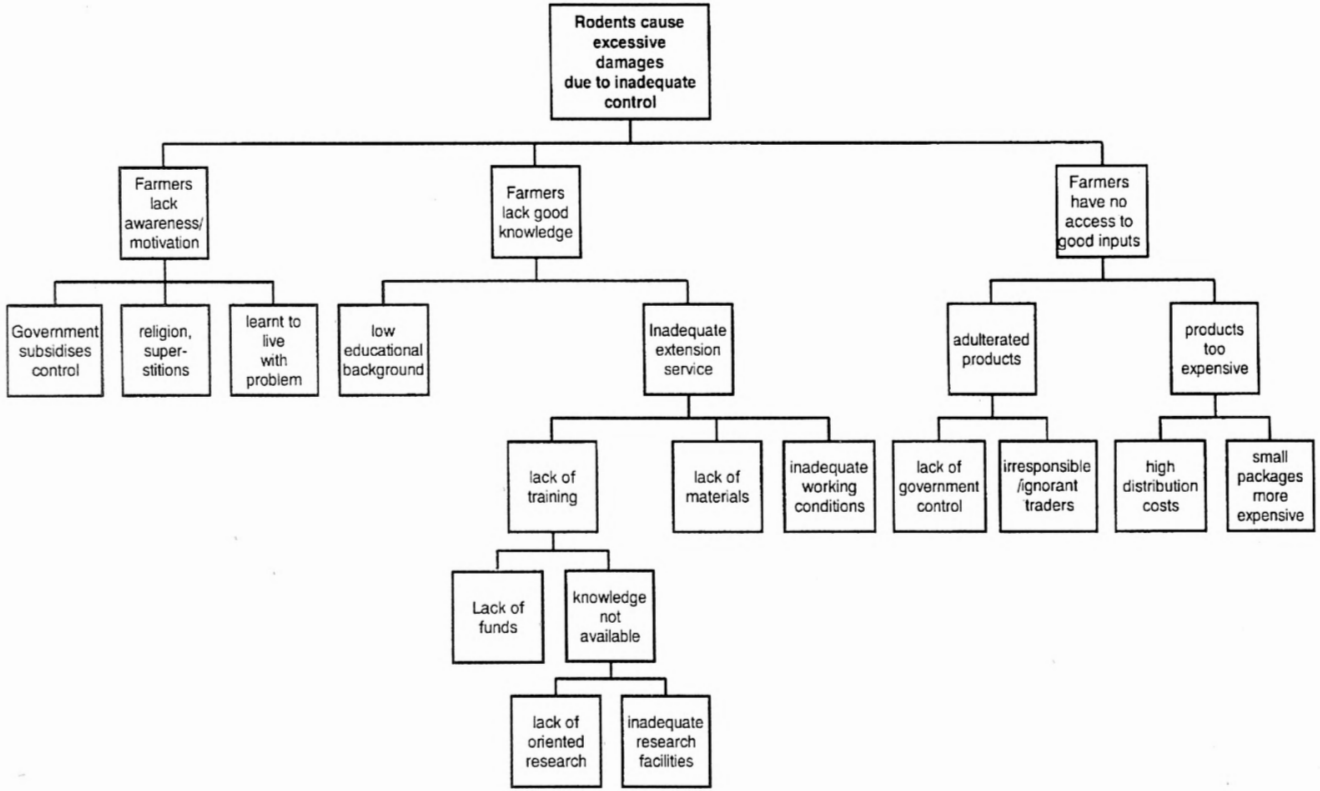

Fig. 1. – Simplified problem tree for generalised rodent control programme

In addition farmers opinion surveys invariably identify further reasons for not controlling rodents (Table 2).

TABLE 2

Reasons for not practising rat control
(adapted from ADHIKARYA & POSAMENTIER, 1987 and reports)

Country	Bangladesh (post-campaign)		Myanmar	Sudan
Year	1983	1984	1987	1991
Percentage not controlling	51	33	76	37
Number not controlling	586	359	183	55
Reasons mentioned	Percentage of farmes not controlling			
No knowledge regarding techniques/do not know	22	24	21	23
Good rodenticides not accessible	12	7	-	-
Rodenticides not available	25	20	<1	9
No rats/no damage	21	18	73	38
Others (no money, no time, etc.)	20	31	5	19

Of course it must be realised that the form and content of such a tree can vary between situations and countries; for discussion purposes it has been simplified slightly. The remainder of the tree details the causes, which would represent the activities of the programme designed to find solutions. Given the scenario presented, a programme concentrating only on designing an efficient rodent control strategy could not expect to reduce damage in the fields of farmers.

SOLUTIONS

In this section some ideas and suggestions are provided to answer some of the identified problems.

Awareness and knowledge

Many farmers are not aware of the benefits of regular rodent control or do not know why they are not carrying out control. Some of the reasons for this apparent lack of awareness and possible solutions are listed in tables 2 and 3. Table 3 serves to impress that some of these barriers have to be removed before the farmer will be involved in control, and this may include more than 30% of the farmer population (Table 2). More details and ideas on

this topic from evaluated rodent programmes can be obtained from ADHIKARYA (1994) and ADHIKARYA & POSAMENTIER (1987).

TABLE 3

Some causes and solutions for the lack of awareness. Suggested solutions/actions

Causes	*Suggested solutions/actions*
The government services have conducted or subsidised rodent control, and consequently farmers do not see the need to look after their own fields.	Lobby government to eliminate subsidies; demonstrate benefits through trials, etc.; use of motivational messages (i.e. «your field is your responsibility»).
Sharecroppers are not willing to control, because benefits go largely to the landowner.	No practical solution unless tenure system is changed.
Rats are seen as a nuisance rather than a threat. The problem is nothing new to farmers, and they have learnt to live with it.	Use of motivational messages creating fear of disease or of insufficient food to feed the family; providing information on benefits.
Lack of knowledge and access to information does not enable them to understand or see the benefits.	Provide information; for example at farmer meetings or through leaflets.
Many extension staff are not sufficiently knowledgeable to provide adequate advice. This leads to a low credibility with farmers.	Improve the extension service.
Many farmers lack faith in the available control methods because of negative past experience with ineffective bait formulations.	Lobby for quality control by government services; influence manufacturers.
Farmers are superstitious: rats are intelligent, take revenge, reincarnations of relatives and friends.	Religious disincentive, it is sinful to be superstitious.

Material inputs for control

The motivation to control rats can quickly wane, if farmers do not have easy access to good quality bait. In general, programmes overcome this handicap by organising the production and distribution on a local scale to demonstrate the benefits. However, this cannot be sustained and attempts should be made to improve the situation during the period of programme activities. Some ideas are listed below.

- Influence the government to eliminate subsidies for rodenticides. It means that manufacturers and distributors have to serve the farmers directly, establishing suitable outlets and packaging.
- The programme can demonstrate to manufacturers the potential market by providing survey statistics.
- Programmes could co-operate with manufacturers through assuring sales by organising and financing national campaigns (see ADHIKARYA & POSAMENTIER, 1987).

- Impress on manufacturers the benefits of improving labels or by-packs (information leaflet sold with item), including information on measures besides the use of poison bait.
- Provide local manufacturers with information on application techniques and good formulations and offer training to their staff.
- Mention reliable and good quality brands in extension activities.
- Introduce quality control procedures for rodenticide baits within the government services.
- Search for alternate distribution channels and make these known to manufacturers.

The above may not solve the problem. However, depending on the situation, close, regular contact and cooperation with sincere manufacturers should eventually lead to an improvement in the accessibility of good quality baits at the level of farmers.

Communication

Communication depends on intermediaries who carry information and motivation to the target group or even block it. Therefore it is necessary to perform an analysis of people and groups who may be involved, benefit or lose from the implementation of a rodent control programme and their possible function and influence. The content, for example the control strategy, should be adapted to the local conditions and be within the scope of farmers. Finally the impact of the programme depends on how the information is packaged, presented and distributed.

Control strategy

Many aspects should be considered when designing a control strategy, for example costs and benefits related to the target crop and level of damage, farmers ability to control at particular times of the year given their commitment to farm management activities and even the climatic conditions. Often too much emphasis is placed on designing a «new» technology aiming at 75% or more effectiveness of control. Farmers may be less willing to adopt such a technology as it involves greater costs, more labour or because it is too complex for them to fully understand it (see also FIEDLER & FALL, 1994). In many situations it would be easier to overcome this resistance by starting with existing farmer practices and modifying these slightly, even if the overall effectiveness may reach 50-60% only at the level of farmers fields. For example in most situations control practices are started at a time when damage by rodents is already obvious and extensive. Yet farmers are aware that damage starts long before harvest time and that efficiency can be increased by initiating control efforts earlier. In rice or wheat this means starting control just before booting stage rather than at the milky stage, which is the normal practice.

The introduction of a control programme should select a crop and time for which implementation is simple and straight forward, relatively inexpensive and benefits are obvious (ADHIKARYA & POSAMENTIER, 1987, p. 202). In Bangladesh, campaigns were conducted during the winter season, when rats inflict obvious damage every year. The control at this time is simple and benefits are certain if farmers have access to good rodenticide baits and follow some simple recommendations. Alternatively or in addition high value

crops (fruits and vegetables) can be selected initially, because for these crops farmers are more likely to spend additional efforts.

Channels of information

The farmer can be reached by many channels of communication, but there are also select groups of people which influence the opinion and attitude of farmers. These groups need to be identified and incorporated into the communication plan to ensure the correct information reaches the farmers.

Extension. In developing countries, extension staff in direct contact with the farmer normally have very little training and facilities. They rely on inter-personal communication skills only. The training on rodent control, if they receive it, is invariably limited to control techniques and a description of the different rodenticides. Often they cannot answer all the questions farmers may pose and therefore loose credibility. In such cases it is unlikely that extension staff can motivate farmers to change their behaviour. Other than information on control techniques, training sessions for staff should include:

- the biology and ecology of rodents,
- didactic techniques (teaching techniques),
- how to use demonstration and extension materials,
- how to organise farmer meetings and follow-up,
- extension strategies, the content of messages, etc.,
- management, organisation and monitoring and evaluation techniques.

The activities of extension staff can be improved, if they are provided with specifically designed and relevant extension and demonstration materials. A comprehensive and attractive brochure will not only motivate them to perform their duties but also ensure a certain degree of quality control in the delivery of technical information (ADHIKARYA, 1994). If these brochures are available, extension staff can develop good rapport with farmers by accessing information together. They can be motivated further if provided with suitable and attractive handouts, which they can pass out to farmers and other groups.

Many programme managers overlook the benefits of such printed materials because of the costs. In reality however these are negligible relative to the total programme costs (ADHIKARYA & POSAMENTIER, 1987).

Agricultural universities and colleges. Agricultural universities and colleges could be approached to determine whether they are interested in receiving courses on rodent biology and control. Course packages could be prepared to be included in their curricula, and if well prepared such courses would continue after the programme itself has terminated. In addition, relevant literature could be provided to these institutes free of charge, and supporting a local post-graduate programme on rodent control will enhance interest in the subject and provide trained professionals. Supporting international networking through active correspondence, workshops and seminars has been proposed several times (*i.e.* QUICK, 1990), but to date no central organisation or institute has shown interest to take on this task.

Efforts to train professionals now will have important spill-over benefits for future generations. Taken in this context, the costs and efforts of educating people are minimal given they are providing an important investment for the future.

School children and clinics. Children can influence their parents – farmers, and will themselves become farmers. They are an easy group to reach and motivate. Well prepared leaflets or small pamphlets could be distributed, teachers could be trained or supplied with demonstration materials or a comprehensive brochure and programme staff can give talks on the subject. Rural health clinics may also be interested since rats transmit various diseases. Training health practioners on the problems caused by rodents and on simple control measures including hygienic practices as well as providing them with handouts for farmers, will sensitise and may motivate farmers because nurses and doctors are respected people. A major chemical company conducted such activities in South America on the subject of pesticide safety, and these activities were well received by farmers and public opinion leaders including the press.

Dealers. Dealers of agricultural inputs (pesticides, fertiliser, etc.) are in most situation accessible to farmers and at the time of the sale is a good opportunity to inform farmers on rodent control practices. Therefore dealers should be included in a training programme and be given handouts for distribution to farmers. Generally, they are interested if they can provide farmers with quality advice because it will improve their business as well as their standing with farmers.

Respected persons. Often respected persons and those in position of power influence the activities in a rural area. It is therefore advantageous to obtain their good will or even cooperation. These influential people can be reached and informed on rodent control activities or campaigns through television, which is their status medium or by direct mailing. They should certainly be included in any major activity, because it is important to them to be informed about planned activities in their area and their involvement will help legitimise the programme.

Contents, messages and materials

Apart from using the most effective channels it is important to select the relevant information, effective messages and materials. Too often farmers are confronted with posters informing them that rats cause damage. This is known and the remaining information on the poster is ignored. Depending on the results of the KAP surveys it may be more relevant to apply peer pressure by «professional farmers control rats early in the season» or arouse fear by «your rats make your children sick». The positioning of messages (*i.e.* on bill boards, radio or timing) also needs a creative and innovative approach as it may compete with other messages. The message and its packaging should also be adapted to the medium, radio or poster, and target, extension staff or farmer. More information on designing, packaging and placing messages can be taken from ADHIKARYA (1994); VAN DEN BAN & HAWKINS (1988).

Pre-tests. Many mistakes could have been avoided and materials could have been more effective, if they had been pre-tested, that is tested with target audience for comprehension, layout and other aspects, before they are printed and distributed or broadcasted.

It is seldom done, because most planners think they know what is needed and know what is understood by the target group or they want to save costs or time. Without exception, it has been my personal experience every time that the graphic artists, extensionists and project managers were astounded at the results from these pre-tests. They are simple, quick and cheap and almost always lead to an improvement of the materials. A very good description of the procedures can be found in HAALAND (1984).

Participation. The benefits of utilising the «participatory approach» should be stressed. Involving the respective target group in the design of media and messages and even control strategies saves time and money and increases the chances that the results are appropriate for the local situation and acceptable to a large section of the target group. Sitting down with three extension agents and a graphic artist for three days, we designed the outline and contents of a manual on plant protection in cotton including visual representations. The manual was to be used by them in farmer meetings. The pre-tests took another three days including travel. Further ideas can be taken from MÜLLER-GLODDE (1991).

PROGRAMME SUSTAINABILITY

In most cases, programmes financed by international agencies are too short to ensure a sustainable impact after termination. The staffing, finances and power structures will hardly have improved or changed to allow activities to continue, which is necessary as rodent management is a ongoing process. Once a major rodent outbreak has subsided, so does the interest in supporting related activities. There are some simple activities which could be included into the programme plan improving the chances of sustainability.

- Institutionalising within the government service a training course package for new extension staff and of repeater courses.
- Providing funds not only for overseas studies, but also at local universities.
- Preparing a course package for agricultural universities and colleges including materials.
- Preparing and distributing self-contained information packages for other groups such as medical services, schools and dealers.
- Providing various institutions including the extension service with sufficient materials such as brochures and handouts to last beyond programme completion.
- Stock libraries of different institutions with reference books.
- Work towards privatising the manufacture and distribution of inputs.
- Lobby the government to charge for services and that these moneys are returned to the respective section to cover replacement costs (i.e. for training, providing materials and expert advice).
- Lobby government to introduce laws and/or regulations such as discontinuing subsidies and controlling the quality of bait.

In addition it would help the cause of rodent control world-wide, if an international organisation co-ordinates various activities in this field. A number of recommendations had been submitted during the IRRI workshop (QUICK, 1990), which are still relevant and should be communicated to decision makers of funding agencies. For example after pro-

gramme completion there should be a follow up by supplying the latest information, enabling participation in seminars and maintaining contacts. This may be achieved by incorporating funding for these activities in the original programme proposals or by creating supra-regional ongoing programmes. The government services and organisations responsible for rodent management activities have to realise that it needs continuous attention and not just at the time when major outbreaks hit the public media.

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