

Failure to infect laboratory rodent hosts with human isolates of *Rodentolepis* (= *Hymenolepis*) *nana*

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Abstract

Confusion exists over the species status and host-specificity of the tapeworm *Rodentolepis* (= *Hymenolepis*) *nana*. It has been described as one species, *R. nana*, found in both humans and rodents. Others have identified a subspecies; *R. nana* var. *fraterna*, describing it as morphologically identical to the human form but only found in rodents. The species present in Australian communities has never been identified with certainty. Fifty one human isolates of *Rodentolepis* (= *Hymenolepis*) *nana* were orally inoculated into Swiss Q, BALB/c, A/J, CBA/CAH and nude (hypothymic) BALB/c mice, Fischer 344 and Wistar rats and specific pathogen free (SPF) hamsters. Twenty four human isolates of *R. nana* were cross-tested in flour beetles, *Tribolium confusum*. No adult worms were obtained from mice, rats or hamsters, even when immunosuppressed with cortisone acetate. Only one of the 24 samples developed to the cysticercoid stage in *T. confusum*; however, when inoculated into laboratory mice the cysticercoids failed to develop into adult worms. The large sample size used in this study, and the range of techniques employed for extraction and preparation of eggs provide a comprehensive test of the hypothesis that the human strain of *R. nana* is essentially non-infective to rodents.

Introduction

Rodentolepis (= *Hymenolepis*) *nana* is the most common cestode infecting humans worldwide (Smyth, 1994). Estimates of the number of human infections range from 20 million (Andreassen, 1998) to 50–75 million (Pawlowski, 1984; Crompton, 1999) worldwide. Humans may also be infected with the rat tapeworm, *Hymenolepis diminuta*, but this is considered rare and usually accidental (Schantz, 1996; Andreassen, 1998) and almost always occurs in children (Tena *et al.*, 1998).

There is confusion in the literature regarding both the nomenclature and host specificity of *R. nana*. A taxonomic revision of hymenolepidids with armed rostellae (hooks present) was proposed by Spasskii in 1954 (Vaucher, 1992) in which *Hymenolepis nana* was reclassified under the genus *Rodentolepis* and this classification is currently recognized by some cestode taxonomists (Czaplinski & Vaucher, 1994). The genus *Hymenolepis* Weinland 1858 now only contains hymenolepidid species with unarmed rostellae (hooks absent) and the type species is the rat tapeworm *Hymenolepis diminuta* (Czaplinski & Vaucher, 1994). Despite the revised nomenclature being adopted by some taxonomists (Spasskii, 1994; Czaplinski & Vaucher, 1994) *Rodentolepis nana* is almost universally referred to as *Hymenolepis nana*.

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Table 1. Inoculation of SPF mice and flour beetles with human isolates of *Rodentolepis* (= *Hymenolepis*) *nana*.

SPF mice or beetles	Species	Number of mice or beetles	Sex	Age	Number of samples tested	Samples yielding cysticercoids (beetles) or adult worms (mice)	Samples yielding adult worms
Swiss Q	Mouse	85	Male	4–5 wks	51	0	0
BALB/c	Mouse	98	Male	4–5 wks	51	0	0
BALB/c nude†	Mouse	5	Male	4–5 wks	1	0	0
Flour beetles	<i>T. confusum</i>	110	ND	ND	11 ^a	1*	0
Flour beetles	<i>T. confusum</i>	130	ND	ND	13 ^b	0	0

^aEggs obtained by NaCl flotation.

^bDirect faecal feeding.

*One sample yielded 73 cysticercoids dissected from five *Tribolium confusum* beetles infected via *ad libitum* feeding of eggs on filter paper.

†Hypothyroid model.

ND, not determined.

in the medical literature. (cf. Arme & Pappas, 1983; Pawlowski, 1984; Smyth & McManus, 1989; Smyth, 1994; Baily, 1996; Schantz, 1996; Lloyd, 1998; Andreassen, 1998).

In Australia, human infections with *Rodentolepis* (= *Hymenolepis*) *nana* are most common in communities in tropical regions. The prevalence of *R. nana* has risen from 20% (Meloni *et al.*, 1993) to approximately 55% in these same communities (Reynoldson *et al.*, 1997) within the last decade. There is uncertainty about the transmission of *R. nana* in these endemic areas where human infection is common. It is not well understood whether the 'strain/species' of *R. nana* present in the north-west of Western Australia is found in rodent and human hosts or whether humans harbour their own form of this parasite. For example, *R. nana* has been described as being infective to both humans and rodents (Yamaguti, 1959). Others have concluded that the rodent form is a subspecies, *R. nana* var. *fraterna*, morphologically indistinguishable from the human form and only infective to rodents (cf. Baer & Tenora, 1970; Pawlowski, 1984; Schantz, 1996; Lloyd, 1998). In the present study, some of these uncertainties were resolved by testing the infectivity of a number of human isolates of *R. nana* in specific pathogen free (SPF) mice, rats and hamsters under varying conditions.

Materials and methods

Source of parasites

A laboratory isolate of *R. nana* was kindly provided by Dr Akira Ito, Gifu University, Japan in two forms: (i) eggs in PBS; and (ii) approximately 30 flour beetles, *Tribolium confusum*, infected with the cysticercoid stage of *R. nana*. The isolate has been passaged through laboratory mice for more than 20 years. Human faecal samples were collected from the north-west region of Western Australia over a period of three years. Positive identification of infection was carried out using the ZnSO₄ flotation technique (Voge, 1970). Eggs were usually extracted from faecal samples using the NaCl flotation method (Voge, 1970) prior to inoculation into specific pathogen free (SPF) mice, rats and hamsters (tables 1 and 2). Other egg extraction techniques used in this study are outlined (see Infection methods tested using human isolates).

Infection of mice and beetles using a laboratory reference strain

The direct and indirect life cycles of *R. nana* were tested in this study using eggs and cysticercoids sent by Dr Ito (Gifu University, Japan). Infected *Tribolium confusum* sent by Dr Ito were killed and dissected using a WILD M7A

Table 2. Cortisone acetate treatment regime in mice, rats and hamsters prior to inoculation with human isolates of *Rodentolepis* (= *Hymenolepis*) *nana*.

SPF strain	Species	Number	Sex	Age	Cortisone treatment	Adult worms obtained
Swiss Q ^a	Mouse	3 ^a /3*	Male	4–5 wks	125 mg kg ⁻¹ ; 5 doses ^a	0
AJ ^a	Mouse	3 ^a /3*	Male	4–5 wks	125 mg kg ⁻¹ ; 5 doses ^a	0
CBA/CAH ^a	Mouse	3 ^a /3*	Male	4–5 wks	125 mg kg ⁻¹ ; 5 doses ^a	0
AJ ^b	Mouse	3 ^b /3*	Male	4–5 wks	125 mg kg ⁻¹ ; 5 doses ^b	0
CBA/CAH ^b	Mouse	3 ^b /3*	Male	4–5 wks	125 mg kg ⁻¹ ; 5 doses ^b	0
Fischer 344 ^c	Rat	3 ^c /3*	Male	4–5 wks	100 mg total in 2 doses ^c	0
Wistar ^c	Rat	3 ^c /3*	Male	4–5 wks	100 mg total in 2 doses ^c	0
DSN ^d	Hamsters	15 ^d	Male	8 wks	25 mg kg ⁻¹ in 2 doses ^d	0

^aCortisone injections days 2, 4, 6, 8, 10, 12 (dose-weight dependent).

^bCortisone injections days -1, 1, 3, 5, 7, 9 (dose-weight dependent).

^cCortisone injections days 4 and 6 (dose-weight independent).

^dCortisone given to all 15 hamsters days -1 and 4.

*Control group received no cortisone.

stereo microscope (WILD Heerbrugg, Switzerland). Cysticercoids were collected into phosphate buffered saline (PBS) and orally inoculated, via stomach tube, into Swiss Quackenbush (Swiss Q) and BALB/c male, 4- to 5-week-old mice. Approximately 11 days later, adult worms were dissected from the small intestine and washed repeatedly in PBS. Eggs were teased from the gravid proglottids from worms obtained in this manner and routinely passaged through mice and beetles. In addition, *R. nana* eggs sent by Dr Ito were stored in PBS at 4°C and were periodically inoculated into mice (19, 23, 26, 62 and 70 days post-collection). This was primarily to provide a positive control of the decline of viability of eggs stored at non-ambient temperatures over a several week period. When mice were inoculated with eggs, rather than cysticercoids, euthanasia and dissection were delayed until day 14 due to the longer pre-patency of infection.

Infection of mice, rats and hamsters using human samples

The SPF mice and rats used in this study (tables 1 and 2) were purchased from the Animal Resources Centre (ARC), Western Australia. The infection trials were conducted at Murdoch University, Western Australia. The hamster infection trial (table 2) was conducted by J.M. Behnke with a human isolate of *R. nana* from our collection. At Murdoch University, *R. nana* eggs were suspended in 0.1 ml phosphate buffered saline, drawn into a 1 ml hypodermic syringe fitted with a blunted 23 gauge needle and 0.5 mm polyethylene tubing then orally inoculated into rodent hosts.

Infection methods tested using human isolates

A variety of egg collection and inoculation methods were tested using human isolates of *R. nana* in both mice and beetles:

1. Oral inoculation of mice, via stomach tube, using eggs extracted by NaCl flotation. The size of each inoculum ranged from 50 to 14 400 eggs (Ferretti *et al.*, 1980; Ito, 1982, 1984; Palmas *et al.*, 1984).
2. Oral inoculation of mice, via stomach tube, using eggs extracted by Ficoll/sodium diatrizoate gradient centrifugation (Lumb *et al.*, 1988) and sucrose gradient methods outlined by Berntzen & Voge (1962), Lethbridge (1971) and Hurd & Burns (1994).
3. Oral inoculation of mice, via stomach tube, using eggs treated by mechanical and chemical 'cracking' with a glass bead and trypsin digestion method outlined by Ito (1975) except that the trypsin (ICN Biomedicals, Ohio, USA) was dissolved in Tyrodes solution (Sigma, St Louis, Missouri, USA) instead of Hanks' balanced salt solution and the glass beads (Sigma, USA) used were smaller (425–600 µm).
4. Spiking of mouse food with eggs for *ad libitum* feeding (Ferretti *et al.*, 1980) except that bread pieces were used instead of ground food pellets provided by the small animal facility, Murdoch University.
5. The pharmacological immunosuppression (Lucas *et al.*, 1980) of mice, rats and hamsters using subcutaneous injections of cortisone acetate (Sigma, USA) as well as the

use of a congenitally hypothyroid mouse model (Ito, 1985).

6. Feeding NaCl-extracted eggs to *Tribolium confusum* (flour beetles) that were pre-starved for 6 days (Nakamura & Okamoto, 1993) on either filter paper (Yan & Norman, 1995) or a latex rubber sheet (Nakamura & Okamoto, 1993).

7. Feeding *T. confusum* (pre-starved for 6 days) directly on infected faeces (Pappas *et al.*, 1995; Yan & Norman, 1995).

Cortisone acetate treatment

Five different strains of SPF mice were used for the experimental procedures including Swiss Q (Thompson, 1972; Seidel & Voge, 1975) BALB/c (Asano *et al.*, 1986; Watanabe *et al.*, 1994; Matsuo & Okamoto, 1995); A/J and CBA/CAH (Ito *et al.*, 1988); two strains of SPF rats: Fischer 344 (Ito, 1983) and Wistar, an equivalent strain to the Rowett rats used by (Ito & Kamiyama, 1987) and SPF hamsters (Ronald & Wagner, 1975).

Mice

Six Swiss Q, A/J and CBA/CAH mice were divided into two equal groups. All mice were weighed prior to the trial to adjust their dose of cortisone accordingly. A sub-cutaneous injection of cortisone acetate (Sigma, USA) at a dose of 125 mg kg⁻¹ (Malinverni *et al.*, 1995) was given to half the group on days 2, 4, 6, 8, 10 and 12 (treatment^a, table 2). The remaining half of each group were used as controls and did not receive any cortisone treatment prior to inoculation with *R. nana* eggs. All six mice in each group were inoculated with 2000 *R. nana* eggs in 0.2 ml PBS on day 0, regardless of their status (controls and test). A slightly modified regime (treatment^b, table 2) was also tried using A/J and CBA/CAH mice whereby mice received their first cortisone dose 24 h prior to inoculation (day -1), followed by five subsequent doses on days 1, 3, 5, 7 and 9. All mice, regardless of their status, were killed and dissected 14 days post-inoculation.

Rats

Six each Wistar and Fischer 344 rats were divided into two equal groups. Half of each group received a sub-cutaneous injection of 50 mg cortisone acetate on days 4 and 6 (Ito & Kamiyama, 1987). All rats (controls and test) were inoculated with 2000 *R. nana* eggs in 0.2 ml PBS on day 0. All rats were killed and dissected 14 days post-inoculation. The same human sample was used to infect all mice and rats used in the cortisone treatment trial, thus eliminating random 'between-sample' variability from the experimental procedure.

Hamsters

Fifteen hamsters were divided equally into five groups. Although it was not possible to use the sample inoculated into the mice and rats, a single human sample was used to inoculate all 15 hamsters. All hamsters received a subcutaneous injection of cortisone acetate (Sigma, USA) at a dose of 25 mg kg⁻¹ 24 h prior to stomach tubing and again on day 4 post inoculation. Each group was inoculated with varying numbers of

eggs. Group 1 received approximately 1860 eggs each; group 2 received 2220; group 3 received 3260 eggs; group 4 received 14 400 eggs and group 5 received 400 eggs. One hamster per group was killed on day 4 to examine the intestine for cysticercoids, another on day 7 to look for immature worms and the remaining hamster on day 14 to look for mature adult worms.

Viability tests

Trypsin digestion

Egg shells were removed from *R. nana* both mechanically and chemically using a modified trypsin digestion method (see point 3, infection methods tested using human isolates).

Nucleic acid dyes

Nucleic acid dye staining techniques used successfully for viability testing of *Giardia* and *Cryptosporidium* spp. by other researchers (Schupp & Erlandsen, 1987; Belosevic *et al.*, 1997), were evaluated for their indicator of viability of *R. nana* eggs extracted from human faeces. Stock and working solutions of fluorescein diacetate (FDA) and propidium iodide (PI) (Sigma, USA) were prepared according to the method outlined by Schupp & Erlandsen (1987) whilst SYTO-9 and hexidium iodide (HI), from the BacLight™ Bacterial gram stain kit (Molecular Probes, Eugene, Oregon, USA), were prepared according to manufacturers instructions. A pellet of *R. nana* eggs was obtained by centrifugation at full speed for 30 s and excess supernatant removed. *Giardia* cultures and *Cryptosporidium* oocysts, maintained routinely in our laboratory, were used as controls (positive and negative) and *R. nana* eggs, heated to 70°C for 30 min, were also used as a negative control. Nucleic acid staining using FDA and PI was carried out on ice for 10 min (Schupp & Erlandsen, 1987) as well as at 37°C for 30 min. Nucleic acid staining using SYTO-9 and HI was carried out for 10, 30 and 60 min at 37°C based on the method outlined by Belosevic *et al.* (1997). A sample of *R. nana* not exposed to nucleic acid dye was also used to check for intrinsic autofluorescence, a phenomenon found to occur in some cells (Steinkamp *et al.*, 2000). Slides of all test and controls were viewed using a confocal laser microscope (BioRad, California, USA) using 488 nm laser at 1% power. A 522Df35 filter was used for FDA and SYTO-9 detection and a 680DF32 filter was used for PI and HI detection.

Results

Infection trials using a laboratory reference isolate in mice and beetles

Oral inoculation of eggs, stored at 4°C, of the Japanese reference strain yielded adult worms from both Swiss Q and BALB/c mice when dissected 14 days post inoculation on all occasions (after 19, 23, 26, 62 and 70 days storage) providing a positive control of viability over time (data not shown). Eggs from Dr Ito's isolate were not tested for viability beyond day 70. *Tribolium confusum* beetles, starved for 6 days, then fed on terminal gravid proglottids obtained from these worms were consistently

infected with cysticercoids upon dissection 3 weeks post-feeding. When mice were inoculated with cysticercoids from beetles sent from Japan, adult worms were consistently obtained whether 3, 4, 6, 7, 10 or 13 cysticercoids were used (data not shown).

Infection trials of human isolates in mice and rats

Of 51 human faecal samples tested in mice and 24 samples cross-tested in beetles, cysticercoid development occurred in *T. confusum* with one sample only (table 1). This faecal sample was stored at 4°C for 23 days prior to egg extraction using NaCl flotation then fed to beetles. Five beetles harboured 73 cysticercoids in total (table 1). Two Swiss Q mice were inoculated with 13 and 25 cysticercoids respectively within 2 h of dissection from the beetles. Two more Swiss Q mice were inoculated three days later with 13 and 22 cysticercoids respectively that had been stored at 4°C since collection. No adult worms developed from these cysticercoids in all four mice (table 1). All remaining human samples failed to yield either cysticercoids in beetles or adult worms in mice, including those inoculated into the hypothyroid BALB/c mice regardless of the methods used for egg collection and inoculation of the hosts (table 1).

Infection trial of mice, rats and hamsters treated with cortisone acetate

All mice and rats inoculated with the same human isolate were not infected with adult worms when dissected 14 days post-inoculation regardless of whether treated with cortisone acetate or not (table 2). Hamsters, from each group, killed and dissected on day 4 post-infection failed to yield cysticercoids from intestinal scrapings. Furthermore, hamsters dissected on days 7 and 14 were not infected with immature or mature worms respectively (table 2).

Viability tests using trypsin digestion and nucleic acid dye staining

Microscopical examination of aliquots of eggs examined after trypsin digestion revealed many *R. nana* eggs with hatched oncospheres (results not shown). When inoculated into mice or fed to beetles, however, no development to cysticercoid stage or adult worm occurred. The use of nucleic acid dyes, FDA, PI, SYTO-9 and hexidium iodide failed to provide an indication of viability of *R. nana* eggs for two reasons. Firstly, it was apparent that *R. nana* eggs are mildly autofluorescent in the absence of the nucleic acid dyes (results not shown). Secondly, it was found that heat-killed *R. nana* eggs exhibited fluorescence with all four nucleic acid dyes (results not shown) rendering the system unworkable for viability discrimination. The protozoan controls used in each system (FDA/PI and SYTO-9/HI) worked according to the published methods outlined, indicating the failure to discriminate between heat-killed and non-treated *R. nana* eggs did not relate to the dyes themselves, or a technical failure of the method.

Discussion

The failure to infect mice, rats and hamsters with the human isolates of *R. nana* collected in Western Australia is consistent with the hypothesis that 'strains' or 'variants' of *R. nana* that are host adapted exist in Australian communities. In an attempt to address this hypothesis, a number of variables were examined in the current study including the use of three different rodent species (mice, rats and hamsters) together with an insect model, supported by large sample sizes in each case. Numerous approaches were tested for their suitability in the infection of the animal models, including the use of several strains, and genetically and pharmacologically modified animals.

Overall, considerable care was taken in handling the eggs to avoid all obvious treatments that could have accounted for poor infectivity. Faecal samples were kept cool and analysed as soon after collection as was practicable. The potential for variation in infectivity rates due to the age of the sample was carefully addressed by using samples that were wide ranging in age. The human samples collected in our survey were always sealed in collection pots, usually immediately, but always within a few hours of collection and stored on ice or refrigerated at 4°C, thus decreasing the risk of long periods of exposure to air, humidity and high temperatures. Quality control included concurrent experiments with a Japanese isolate of up to 70 days of age (stored at 4°C) which yielded adult worms when inoculated into mice on all occasions, suggesting that eggs remain viable for long periods if storage conditions are appropriate. In this study, oral inoculation of eggs into mice and beetles, including the direct feeding of faeces to beetles, was carried out using human faecal samples that ranged in age from 1 h (beetles only) through to 72 days old (mice and beetles). Cysticercoids did not develop in the beetles in all but one sample, nor did adult worms develop in any rodent host, regardless of their immune and genetic status. The development of *R. nana* eggs from one human sample to cysticercoid stage in *T. confusum* is a positive indication of viability. Reports in the literature regarding egg viability are contradictory. Maki & Yanagisawa (1987) suggest that the infectivity of eggs decreases after 17 h exposure to air. In contrast, Pawlowski (1984) report a survival of eggs in the environment up to 10 days. Kennedy (1983) found that eggs of *H. diminuta* can survive and remain infective in rat faeces for up to 60 days when conditions are optimal.

The extraction techniques employed in this study are unlikely to have damaged the *R. nana* eggs. Although the use of NaCl concentration has been criticized by some researchers who believe the procedure may reduce the infectivity of *R. nana* eggs (Yan & Norman, 1995; Ferretti *et al.*, 1980), Thompson (1972) repeatedly succeeded in infecting albino mice with *R. nana* eggs obtained by NaCl flotation and other researchers routinely use NaCl extraction procedures for obtaining *R. nana* eggs for testing in mice (Nakamura & Okamoto, 1993). In addition, Hurd & Burns (1994) used a combination of saturated NaCl and sucrose to extract *H. diminuta* eggs from faeces and successfully infected laboratory rats. Moreover, the only sample which developed to cysticercoid

stage in beetles in this study was extracted using saturated NaCl flotation.

Berntzen & Voge (1962) first suggested that the preparation of 'shell-free' *R. nana* eggs increases their infectivity and this has been used routinely for obtaining eggs for inoculation into beetle and rodent hosts (cf. Ito, 1977, 1982, 1984). In our laboratory the egg shells were removed both mechanically and chemically using the methods outlined by these researchers with some modification (see point 3 – Infection methods tested using human isolates). Despite observing many *R. nana* eggs with hatched oncospheres, these did not develop to the cysticercoid stage or adult worm when fed to beetles or inoculated into mice.

The numbers of cysticercoids inoculated into mice in our study were consistent with other researchers who have reported successful infection to adult worm stages with 1–20 cysticercoids (Lucas *et al.*, 1980; Ito *et al.*, 1988; Kumazawa, 1992). Furthermore, in the present study, the consistent development of cysticercoids, obtained from the Japanese control isolate, into adult worms when inoculated into laboratory rodents indicated that the storage of cysticercoids in PBS prior to inoculation did not adversely affect the viability of the cysticercoids. This is consistent with the methods outlined by Nakamura & Okamoto (1993) who store their cysticercoids in physiological saline.

Based on previous research (Lucas *et al.*, 1980; Ito *et al.*, 1988; Asano *et al.*, 1992; Maki & Ito, 1998) several different susceptible rodent hosts were used in the inoculation trials in this study. However, the use of these highly susceptible mice, even when further immunosuppressed with cortisone, and the use of hypothymic rodent hosts did not result in the development of eggs to either cysticercoid or adult worm stages *in vivo*.

The lack of development of 23 of the 24 human samples of *R. nana* eggs tested in *T. confusum* beetles to cysticercoid stage in this study is unlikely to be attributable to factors such as variability in the genetics, sex and nutritional status of the beetle host. Given that Yan & Norman (1995) found that two beetle species, *T. confusum* and *T. castaneum*, varied in their susceptibility to *H. diminuta* as well as showing significant among-strain and between-sex variation, only one species, *T. confusum*, was used in our study at all times. In addition, there is evidence that male beetles harbour significantly greater numbers of cysticercoids than female beetles when infected with *H. diminuta* eggs (Pappas *et al.*, 1995). Whilst the sex of the beetles employed in this study was not determined, the strain is routinely used in our laboratory for the maintenance of *H. diminuta* suggesting that the sex ratio of males to females is sufficient for routine passage of this parasite. Furthermore, this strain of beetle is routinely starved for 4–5 days prior to inoculation with *H. diminuta* eggs in our laboratory. In experiments conducted with *R. nana*, however, beetles were routinely starved for 6 days, as proposed by Nakamura & Okamoto (1993), suggesting the failure to demonstrate infection with *R. nana* in beetles in this study was not due to insufficient starvation times. The failure of human isolates to develop to cysticercoid stage in beetles may, therefore, be

explained by a progressive loss of adaptation of the Australian human isolates to these intermediate hosts. In light of this suggestion, the development of one of the 24 human samples to cysticercoid stage in beetles is difficult to explain.

It is not entirely clear whether rodent species play a role in the transmission of *R. nana* in Australia or whether they harbour their own variant species/strain. In view of the reliability of the experimental protocols being controlled by the use, in parallel, of eggs and cysticercoids from a mouse strain of *R. nana* combined with the use of a large sample size in this study, the failure to infect mice, rats and hamsters with 51 human isolates of *R. nana* provides supportive evidence towards the hypothesis that a human variant of *R. nana* exists in Australia.

A poor understanding of the dynamics of transmission of a parasite undermines both short and long term control strategies which can only be effective when accurate epidemiological information is obtained (Holland *et al.*, 1996). Identifying the risk factors within communities, from detailed epidemiological data, is likely to be the most cost efficient for implementing control strategies (WHO, 1996). In addition to the biological data provided here, the genetic characterization of *R. nana* would help to clarify the epidemiology of infections in local, endemic, communities and in rodent populations and would provide the springboard for a strategic approach to the implementation of long-term control programmes.

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