A survey of zoonotic pathogens carried by Norway rats in Baltimore, Maryland, USA

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(Received 9 November 2006; first published online 15 January 2007)

SUMMARY

Norway rats (Rattus norvegicus) carry several zoonotic pathogens and because rats and humans live in close proximity in urban environments, there exists potential for transmission. To identify zoonotic agents carried by rats in Baltimore, Maryland, USA, we live-trapped 201 rats during 2005–2006 and screened them for a panel of viruses, bacteria, and parasites. Antibodies against Seoul virus (57.7%), hepatitis E virus (HEV, 73.5%), Leptospira interrogans (65.3%), Bartonella elizabethae (34.1%), and Rickettsia typhi (7.0%) were detected in Norway rats. Endoparasites, including Calodium hepatica (87.9%) and Hymenolepis sp. (34.4%), and ectoparasites (13.9%, primarily Laelaps echidninus) also were present. The risk of human exposure to these pathogens is a significant public health concern. Because these pathogens cause non-specific and often self-limiting symptoms in humans, infection in human populations is probably underdiagnosed.

INTRODUCTION

Norway rats (Rattus norvegicus) are prevalent in urban environments and pose a threat to public health, both through their destructive behaviour and by serving as reservoirs for pathogens that can be transmitted to humans. A survey of residents of Baltimore, Maryland found that nearly two-thirds of respondents (64%) observed rats in streets and alleys, 6% saw rats inside residences, and 1.2% had experienced a rodent bite in their lifetime [1]. Although Norway rats are reported to be hosts for a large number of pathogens [2], a comprehensive survey of pathogens carried by rats in an urban setting has not been conducted. In urban environments, humans and rats live in close proximity and the potential for spillover of zoonotic agents poses a public health concern that has rarely been evaluated. To identify and assess the prevalence of zoonotic agents carried by
rats in an urban environment, we conducted a survey of pathogens carried by Norway rats in Baltimore, Maryland in 2005–2006, including assessment of the prevalence of Seoul virus, hepatitis E virus (HEV), lymphocytic choriomeningitis virus (LCMV), Leptospira interrogans, Bartonella elizabethae, and Rickettsia typhi by serological analyses, and the presence of Hymenolepis sp. and Calodium (syn. Capillaria) hepatica.

**METHODS**

**Wild-caught rats**

Adult male and female *R. norvegicus* were live-trapped (Tomahawk Trap Co., Tomahawk, WI, USA) from 20 locations in neighbourhoods in East Baltimore, Maryland. Rats were trapped from April 2005 to April 2006. The sampling strategy was designed to trap similar numbers of rats in each season to account for possible seasonal variation in pathogen prevalence. All trapping locations were in urban areas in alleys behind residential dwellings. Traps were baited with peanut butter and set at locations ~1–2 h before sundown. Details of sampling procedures have been previously described [3]. Rats were collected and processed the next morning. Rats were euthanized using CO$_2$, weighed, sexed, and bled by cardiac puncture. Serum was stored at $-80^\circ$C until serological analysis. Each rat was examined for ectoparasites using a fine comb. Faecal and caecum content samples were collected for helminth ova analysis. The Johns Hopkins Animal Care and Use Committee (protocol no. RA05H6) approved all procedures described in this study.

**Serological analyses**

**Seoul virus**

Anti-Seoul virus IgG was measured by ELISA as previously described [4]. Microtitre plates were coated with lysate from Vero E6 cells infected with Seoul virus or from uninfected Vero E6 cells. Sera from experimental and control rats were diluted 1:100 and added to plates in duplicate. Secondary antibody [alkaline phosphatase-conjugated anti-rat IgG; Kirkegaard and Perry Laboratories (KPL), Gaithersburg, MD, USA] was added and developed with $p$-nitrophenylphosphate substrate buffer. Optical density (OD) was measured at 405 nm and the average OD for each set of infected Vero E6 duplicates was subtracted from the average OD for each set of infected Vero E6 duplicates. Samples were considered positive if the average adjusted OD was $>0.100$ nm.

**HEV**

Anti-HEV IgG was measured by ELISA as previously described [5, 6]. Microtitre plates were coated with ORF2 antigen (0.1 $\mu$g/well). After blocking, sera from test and control rats were diluted 1:100 and added to plates in duplicate. Secondary antibody [horseradish peroxidase (HRP)-labelled goat anti-rat IgG (KPL)] was added and developed with azino-dietylbenzoyl-sulfonate (ABTS) substrate. OD at 405 nm was measured and the cut-off was established for each test from internal controls; throughout this study the cut-off OD averaged 0.370.

**LCMV**

Serology samples were submitted to a commercial laboratory (BioReliance SM, Rockville, MD, USA). Antigen from LCMV strain CA1371 (obtained from Wallace P. Rowe) grown in Vero E6 cells was used for the ELISA assays. Tests positive by ELISA were confirmed by IFA.

**Leptospira sp.**

Anti-*L. interrogans* IgG was measured by ELISA as previously described [7]. Microtitre plates were coated with sonicated antigen prepared from cultures of *Leptospira* serovars Tarassovi and Pyrogenes (0.1 $\mu$g/well). Sera from test and control rats were diluted 1:100 and added to plates in duplicate. Secondary antibody (peroxide-conjugated anti-rat IgG (Sigma, St Louis, MO, USA) was added and developed with tetramethylbenzidine (TMB). Following termination of the enzyme-substrate reaction with H$_2$SO$_4$, the OD was measured at 450 nm. The OD was standardized by dividing the sample OD by the OD of the pooled negative controls and samples were considered positive when the standardized OD was $>2.4$. *Leptospira* serogroups were identified by a microagglutination test (MAT) with 10 serotypes of *L. interrogans* as previously described [7]. The end-point titre was determined as the highest serum dilution (minimum 1:20) showing agglutination of at least 50% of the cells.

**Rickettsia typhi**

Anti-*Rickettsia* sp. IgG was measured by IFA as previously described [8]. *R. typhi* (Wilmington strain)
grown in DH-82 cells were dotted onto slides. Sera from test and control rats were diluted to 1:32 and added to slides. Secondary antibody [FITC conjugated goat anti-rat IgG (KPL)] was added and slides were mounted with a glass coverslip over a glycerol-based mounting medium. Sera were determined to be positive when discrete, fluorescent organisms were visible. Sera that were positive at 1:32 were retested at 1:64 and 1:100. To determine cross-reactivity, slides were visible. Sera that were positive at 1:32 were retested at 1:64 dilution. For all antigens, a positive serum was defined as a titre of ≥1:64.

Bartonella elizabethae

Anti- B. elizabethae IgG was measured by IFA. B. elizabethae bacteria (strain F9251) grown in Vero E6 cells were dotted onto poly-l-lysine-coated slides, air dried, and fixed in 1% paraformaldehyde for 1 h. Plates were washed with PBS (three times for 5 min) in between each step. Following blocking with PBS + 10% FBS, sera from test and control samples were diluted 1:50 in PBS + 2% FBS and 15 μl dotted on the appropriate well. Slides were incubated for 30 min at 37 °C and secondary antibody [FITC conjugated goat anti-rat IgG (H + L, KPL)] was diluted 1:100 in PBS and added to each well. Slides were incubated in the dark for 30 min at 37 °C, dried, and mounted with a coverslip after adding a small drop of glycerol to each well. Sera were determined to be positive when discrete, fluorescent organisms were visible.

Calodium hepatica

C. hepatica adults and eggs were visible as yellowish-white lesions in rat livers and a subset of adults were verified by light microscopy (100 × magnification).

Hymenolepis sp. faecal and caecum content floats

Faecal and caecum content samples were homogenized in zinc sulphate buffer (400 g/l) in glass test tubes and filled to the brim with buffer. A coverslip was placed on top for 15 min and transferred to a slide for microscopic evaluation. Both H. nana and H. diminuta ova were identified, but were not differentiated in data records. Helminth ova identification was conducted after initiation of this study; thus fewer rats were examined for helminth infection compared with serological analyses.

Statistics

Differences in pathogen prevalence by various demographic strata included age, sex, seasonality, and pregnancy status and were evaluated by χ² or Fisher’s exact tests. Weight was used as a correlate of age as follows: juveniles were < 200 g (n = 31), young adults were 200–399 g (n = 71), and adults were ≥400 g (n = 98). Rats over 200 g were sexually mature as indicated by the decent of testes in males and development of vaginal openings in females. Seasons were defined as: winter (December–February), spring (March–May), summer (June–August), and autumn (September–November). Correlational analyses were conducted using Pearson product moment. Comparisons were considered statistically significant at P < 0.05.

RESULTS

Prevalence of zoonotic pathogens

Prevalence of antibody or rodent-borne pathogens is presented in decreasing order (Table). The most common pathogen was the nematode C. hepatica (87.9%, 176/201). Antibodies against HEV (73.5%, 144/196) and Seoul virus (57.7%, 116/201), as well as L. interrogans (65.3%, 124/190), were detected in over half of the Norway rats tested. The tapeworms H. nana or H. diminuta were observed in more than one-third of rats (34.0%, 55/162). Seroprevalence for ectoparasite-borne bacteria was highest for B. elizabethae (34.1%, 63/197), followed by R. typhi (7.0%, 14/201). Antibodies against LCMV were not detected in a subset of rats that were tested (0/48).

The spiny rat mite (Laelaps echidninus) was the most prevalent ectoparasite (12.4%, 25/201). Two cat fleas (Ctenocephalides felis) and three tropical rat mites (Ornithonyssus bacoti) also were collected during the summer months. A representative selection of serum samples that tested positive for L. interrogans (n = 15) were tested by MAT and showed specific titres against L. copenhageni (Icterohaemorrhagiae serogroup).

Body size

The presence of antibodies against Seoul virus, HEV, and L. interrogans significantly increased with age class (χ² = 53.67, 2, P < 0.001; χ² = 57.25, 2, P < 0.001; χ² = 48.26, 2, P < 0.001, respectively) (Fig). Prevalence of C. hepatica was significantly higher in young adults
and adults compared with juveniles ($\chi^2=6.33, 2, P=0.042$) (Fig.). Seroprevalence of *B. elizabethae* and *R. typhi* as well as the prevalence of ectoparasites and *Hymenolepis* sp. did not differ according to age.

### Sex

Comparable numbers of males ($n=105$) and females ($n=96$) were collected and there was no difference in the numbers of males and females trapped by age class ($P>0.05$). The prevalence of *Hymenolepis* sp. was higher in males compared with females ($\chi^2=6.46, 1, P=0.011$). Conversely, the prevalence of antibodies against *L. interrogans* was higher in females compared with males ($\chi^2=4.52, 1, P=0.033$). Sex differences were not observed in association with antibodies against Seoul virus, HEV, *B. elizabethae*, or *R. typhi*, or the presence of *C. hepatica* or ectoparasites ($P>0.05$ for all).

### Seasons

Attempts were made to collect similar numbers of rats during each season, but autumn was especially rainy, so trapping success was reduced during this time period ($n=20$). Numbers of rats trapped during other seasons, i.e. winter ($n=64$), spring ($n=54$), and summer ($n=63$) were otherwise comparable. Seasonal differences were observed for *Hymenolepis* sp.: the prevalence was significantly lower in spring (17.4%) compared with summer (45.5%), autumn (40.0%) and winter (38.1%) ($\chi^2=8.38, 3, P=0.04$). The prevalence of spiny rat mites was significantly higher in summer and autumn (23.8% and 25%, respectively) compared with winter and spring (4.7% and 3.7%, respectively, $\chi^2=21.08, 3, P<0.001$). There were no significant seasonal patterns in the prevalence of Seoul virus, HEV, *L. interrogans*, *B. elizabethae*, *R. typhi*, and *C. hepatica* observed.

### Pregnancy

Thirty-three percent (32/96) of the female rats were pregnant at the time of trapping, with the highest rate of pregnancy during winter (52.0%) and the lowest during summer (16.1%). Pregnant females were
significantly more likely to have antibody against Seoul virus than were non-pregnant females \((\chi^2 = 3.94, 1, P = 0.047)\). Age was a confounding factor and after stratification by age class, the effect of pregnancy on infection no longer exists among adult females (for young adults, Fisher’s exact test, \(P > 0.05\) and for adults, \(\chi^2 = 1.28, P > 0.05\)). There was no effect of pregnancy on the presence of antibodies against HEV, \(L.\) interrogans, \(B.\) elizabethae, or \(R.\) typhi, or the presence of \(C.\) hepatica, Hymenolepis sp., or ecto-parasites \((P > 0.05\) for all).

**Correlations**

There was a correlation between prevalence of \(L.\) interrogans and HEV \((r = 0.36, P < 0.001)\). No significant correlation existed between Seoul virus infection and \(L.\) interrogans or HEV infection \((r < 0.1, P > 0.05)\); in fact, the presence of antibodies against Seoul virus was not correlated with the likelihood of being infected with any of the other pathogens tested \((P > 0.05\) for all tests).

**DISCUSSION**

Norway rats serve as reservoirs for a variety of zoonotic pathogens. The panel of pathogens was selected because these organisms have been identified in both humans and rats in urban environments and resources were readily available for testing in rats. Increasing age-related seroprevalence of Seoul virus, HEV, \(L.\) interrogans, and \(C.\) hepatica in rats has been previously documented and probably reflects an increased probability of encountering pathogens with age \([4, 9–11]\). The impact of pregnancy on infection has not been reported in wild rat populations and seems to have little effect on seroprevalence of viruses and bacteria or the prevalence of helminths. Pregnant females, however, were more likely to be infected with Seoul virus than were non-pregnant females. The absence of sex differences in infection with Seoul virus and \(C.\) hepatica is consistent with previous studies \([4, 11]\). Reasons for male-biased Hymenolepis sp. infection and female-biased \(L.\) interrogans infection are unknown. Taken together, the effects of sex-related hormones, including testosterone, oestradiol, and progesterone, on the prevalence of infections in wild-caught rats may be masked by social and/or environmental factors that affect exposure \([12, 13]\).

Seasonal effects were observed only for parasites (i.e. Hymenolepis sp. and spiny rat mites). Consistent with previous data, seasonal patterns in Seoul virus, \(L.\) interrogans, and \(C.\) hepatica were not observed \([4, 11, 14]\). Seroprevalence is not expected to show seasonal fluctuations because antibodies remain in circulation whether rats are chronically infected or have cleared the infection. Conversely, seasonal changes in the prevalence of pathogens may be pronounced because differences in the social behaviour, habitat, and environment can affect parasite populations as well as the likelihood of coming in contact with pathogens.

Pathogens that are transmitted by similar routes would be expected to infect the same individuals. Presence of antibodies against \(L.\) interrogans and HEV were correlated \((r = 0.36, P < 0.001)\) and both pathogens are transmitted among rat populations by ingestion of contaminated urine or faeces during social contact. Although Seoul virus also is transmitted during social contact, no correlation existed between presence of antibodies against Seoul virus and \(L.\) interrogans or HEV \((P > 0.05)\).

Seroprevalence of Seoul virus has been reported to be \(~50\%\) in rats in Baltimore, Maryland \([15, 16]\). Our data are consistent, as the seroprevalence was 57.7% in this survey. Rats are persistently infected for the duration of their lives and do not show signs of disease, reduced fertility, or mortality from infection \([17]\). Rodents release infectious virus in excrement and saliva and transmission is hypothesized to occur through inhalation of aerosolized virus in urine and faeces and passage of virus in saliva during aggressive encounters \([18]\). Evidence for zoonotic transmission of Seoul virus has been documented in Baltimore City populations \((0.25\%\) and \(0.74\%\) seroprevalences) as well as in homeless populations in Los Angeles, California \((0.5\%)\) \([1, 19, 20]\). Disease manifestations are acute cases of haemorrhagic fever with renal syndrome (HFRS) and although the symptoms are relatively non-specific, infection is associated with hypertensive renal disease \([21]\). Norway rat-borne hantavirus infection occurs globally and although the mortality is low \((<5\%)\), no effective treatment exists.

A previous study over half a century ago reported that 50–5% of wild-caught rats in Baltimore had antibodies against \(L.\) interrogans (Icterohaemorrhagiae serogroup), which is consistent with our reported 65.3% \([14]\). Rats become chronically infected following contact with contaminated urine through a wound or mucous membranes. Transmission to
humans occurs in the same manner, often in contaminated water or directly through percutaneous exposure (i.e. through cuts on the feet) in alleys, but has also been documented as being transmitted by rat bites [22, 23]. Zoonotic transmission has been demonstrated in Baltimore (16% seroprevalence) as well as in Detroit (31%) [22, 24]. Pathology in rats is considered to be subclinical. In humans, clinical manifestations are usually non-specific and self-limiting, but if left untreated, the disease can progress to Weil’s disease, which is characterized by jaundice, acute renal failure, and possible death [22]. Antigens against HEV have been reported in Norway rats in Baltimore (77%), as well as in other urban centres, including Los Angeles (73-1%) [9, 20]. The current data collected in Baltimore are consistent with these findings, as 73% of the Norway rats were seropositive for HEV. Rodents and humans are primarily infected via the faecal–oral route and the self-limiting infection causes no apparent pathology in rats. Although HEV often causes subclinical disease in humans (<1% mortality rate), it can be particularly lethal for women exposed during their third trimester of pregnancy (≥20% mortality) [25]. Seroprevalence in Baltimore blood donors is reported to be 21.3%, which is similar to other urban centres (i.e. Los Angeles), but few clinical cases have been diagnosed in the United States [20, 26]. The mechanism of exposure remains unknown [25].

*B. elizabethae* has been isolated from Norway rats in Baltimore (10.6%) as well as from rats in other urban centres, including New Orleans (56.4%) [27]. In the present study, 39% of the trapped rats had detectable antibodies against *B. elizabethae*. Exposure to *Bartonella* sp. causes persistent circulating bacteraemia without pathology in rats. *B. elizabethae* is a newly emerging infection in humans and although often self-limiting, without treatment can cause potentially fatal endocarditis [27]. Antibodies to *B. elizabethae* have been found in inner-city injection drug users in Baltimore (33%) [28]. The reservoir for *B. elizabethae*, as well as the mechanism of transmission, remains unknown. Rat fleas (*Xenopsylla cheopsis*) or other ectoparasites may act as vectors for human transmission, so proximity to rats and their ectoparasites may be a risk factor for *B. elizabethae* infection [29].

A serological survey in Los Angeles reported higher prevalences of *R. typhi* (25.9%) in Norway rats compared with our data (7%) [20]. A subset of the serum samples (n=90) were previously screened for *R. typhi* and the prevalence remained the same even as additional rats were included [8]. Screening for *R. typhi* rarely occurs in the absence of an outbreak, therefore little data is available for baseline seroprevalence in rats in urban centres. Transmission among rodents or from rodents to humans requires an ectoparasite vector, typically the rat or cat flea (*X. cheopsis* or *C. felis*). Fleas do pose a potential threat, as do blood-sucking mites (i.e. the tropical rat mite *O. bacoti*). Two tropical rat mites were collected from seropositive rats and were tested for the presence of *R. typhi* DNA, but were negative. *R. typhi* is the aetiological agent of murine typhus, an often self-limiting febrile illness which can cause complications in immunocompromised populations (1% mortality rate).

The prevalence of *Hymenolepis sp.* in urban centres has not been previously reported and we reported a prevalence of 34.0% in Baltimore. Both dwarf tapeworms (*H. nana*) and rat tapeworms (*H. diminuta*) are transmitted by insect vectors; however, infectious ova from *H. nana* can also be spread by the faecal–oral route. Humans and other animals become infected when they eat material contaminated by infected insects or faeces. Both rat and human infections are usually subclinical, but symptoms such as gastrointestinal system discomfort and diarrhoea, can ensue during heavy infections.

A previous study reported an 87.4% prevalence of *Calodium hepatica* in Norway rats in residential areas of Baltimore, which is consistent with the 87.9% prevalence reported in this survey [11]. *C. hepatica* is primarily transmitted by predation and ingestion of ova in the host liver. Release of ova that embryonate in the surrounding environment can pose a threat if ingested. Human cases of capillariasis are rare, but can result in liver damage and fatality [11].

Previous data revealed that LCMV is detected in 4.7% of inner-city Baltimore residents and has been found in house mice (*Mus musculus*) in Baltimore (9.0%) [1, 30]. Natural LCMV infection has not been reported in Norway rats. No rats tested positive for LCMV; therefore we conclude that rats do not act as a vector for LCMV infection in humans.

In summary, this survey of zoonotic pathogens provides important background seroprevalences and prevalences in the absence of outbreaks. Interpretation of serological analyses has some limitations, including potential cross-reactivity, sensitivity, and specificity limitations. The mode of transmission, prevalence in rodent populations, and duration of infection influence the risk of zoonotic transmission.
to humans. The presence of antibody does not necessarily indicate an ongoing infection; therefore, the duration of infection (i.e. acute vs. chronic) is an important factor in considering risk of transmission. For example, a pathogen that is aerosolized, is highly prevalent in rat populations, and chronically infects rats would pose a high risk of transmission to humans. Of the pathogens evaluated, the highest risk would pose a high risk of transmission to humans. Of the pathogens evaluated, the highest risk would pose a high risk of transmission to humans. Of the pathogens evaluated, the highest risk would pose a high risk of transmission to humans. Of the pathogens evaluated, the highest risk would pose a high risk of transmission to humans. Of the pathogens evaluated, the highest risk would pose a high risk of transmission to humans. Of the pathogens evaluated, the highest risk would pose a high risk of transmission to humans. Of the pathogens evaluated, the highest risk would pose a high risk of transmission to humans. Of the pathogens evaluated, the highest risk would pose a high risk of transmission to humans. Of the pathogens evaluated, the highest risk would pose a high risk of transmission to humans. Of the pathogens evaluated, the highest risk would pose a high risk of transmission to humans. Of the pathogens evaluated, the highest risk would pose a high risk of transmission to humans. Of the pathogens evaluated, the highest risk would pose a high risk of transmission to humans. Of the pathogens evaluated, the highest risk would pose a high risk of transmission to humans. Of the pathogens evaluated, the highest risk would pose a high risk of transmission to humans. Of the pathogens evaluated, the highest risk would pose a high risk of transmission to humans. Of the pathogens evaluated, the highest risk would pose a high risk of transmission to humans. Of the pathogens evaluated, the highest risk would pose a high risk of transmission to humans. Of the pathogens evaluated, the highest risk would pose a high risk of transmission to humans. Of the pathogens evaluated, the highest risk would pose a high risk of transmission to humans. Of the pathogens evaluated, the highest risk would pose a high risk of transmission to humans. Of the pathogens evaluated, the highest risk would pose a high risk of transmission to humans. Of the pathogens evaluated, the highest risk would pose a high risk of transmission to humans. Of the pathogens evaluated, the highest risk would pose a high risk of transmission to humans. Of the pathogens evaluated, the highest risk would pose a high risk of transmission to humans. Of the pathogens evaluated, the highest risk would pose a high risk of transmission to humans. Of the pathogens evaluated, the highest risk would pose a high risk of transmission to 

ACKNOWLEDGEMENTS

We thank Darren Kaw for help with trapping the rats during summer 2005 and Andrew Glenn and Bruce Baldwin for with help in identification of helminth ova, Dr A. D. Loftis for her help with rickettsial serology, and Ronald Engle (NIAID) for performing the anti-HEV tests. Financial support was provided by NIH grant R01 A1054995 (S. L. K.) and NSF grant EF0525751 (G. E. G.).

DECLARATION OF INTEREST

None.

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