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Sero-epidemiological study of the presence of hantaviruses in domestic dogs and cats from Belgium

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ABSTRACT

Hantaviruses are worldwide rodent-borne pathogens infecting humans and other animals mainly through inhalation of aerosols contaminated with rodent excreta. Few data are available on hantavirus serology and geographical distribution in dogs and cats. We therefore screened sera from pet dogs ($N = 410$) and cats ($N = 124$) in two regions of Belgium, using IgG ELISA and IFA. We analysed the effect of the owner's address as well as pet gender and age on hantavirus status. Hantavirus antibodies were found in both species with a significantly higher seroprevalence in cats than in dogs (16.9% vs. 4.9%, $P = 0.001$). More dogs were infected in highly forested southern Belgium (harbouring more rodents) than in northern Belgium (10.5% vs. 3.0%, $P = 0.002$). In the south, hantavirus sero-positive cats were found in more densely forested localities than sero-negatives ones were ($P = 0.033$). These results are consistent with the ecological variations of hantavirus risks in humans.

1. Introduction

Emerging infectious diseases represent an increasing concern worldwide (Jones et al., 2008). The screening for zoonotic pathogens in potential animal hosts and in domestic animals is a crucial step to be prepared for new emerging threats (Zeier et al., 2005). Pets are indeed a key issue as they are in close proximity to humans and so numerous. In 2004 in Belgium, the 10×10^6 inhabitants possessed more than 1×10^6 dogs and 2×10^6 cats (http://statbel.fgov.be). These domestic dogs and cats can repeatedly be in contact with rodents or rodent contaminated areas, potential sources of hantaviruses.

Rodent-borne hantaviruses belong to the Bunyaviridae family. They are carried worldwide by Muroid rodents (and insectivores, Arai et al., 2008. Each hantavirus is mainly associated with a distinct reservoir species but spillover infections in other rodent species are reported (Klingström et al., 2002). Bites represent one important means of intraspecific transmission, at least in rats (Glass et al., 1988). However indirect transmission is not negligible as, under the optimal conditions, Puumala hantavirus (PUUV) can survive for up to 2 weeks outside of its host (Kallio et al., 2006). In the natural reservoirs, hantaviruses establish a persistent infection, and the host develops a strong neutralizing antibody response (Chu et al., 1994). Yet few detrimental effects have been described in the reservoir species (Kallio et al., 2007).

Despite this, more than a third of the 43 reported serotypes can cause disease in humans: hemorrhagic fever with renal syndrome (HFRS) in Europe and Asia, and hantavirus pulmonary syndrome on the American continent (Lee and van der Groen, 1989). HFRS is caused by eight serotypes. Its symptoms include fever, abdominal and back pain as well as renal dysfunction, sometimes with hemorrhagic manifestations (Vapalahti et al., 2003). Nephropathia epidemica (NE), the milder form of HFRS, caused by PUUV hantavirus, is present in North-Western Europe (including Belgium). Humans are infected by inhalation of aerosols contaminated with excreta from infected rodents. Worldwide, about 150,000–200,000 people are annually hospitalised for HFRS, mainly in Asia (Vapalahti et al., 2003).

In spite of this incidence, little is known about the presence of hantavirus in non-rodent mammals. Apart from insectivores, hantaviruses are known in chiropters (bats, Kim et al., 1994) and artiodactyls (even-toed ungulates, Ahlm et al., 2000). In 20 species of non-human primates, experimental infection leads to an antibody reaction (Yanagihara et al., 1988). As for carnivores, *Felis catus* (cat) was found positive (2.9–9.6%, Xu et al., 1987; Nowotny, 1994; Leighton et al., 2001) as well as *Canis familiaris* (dog, 4.7%, Malecki et al., 1998) and *Vulpes vulpes* (red fox, 2.4%, Escutenaire et al., 2000b). Non-rodent infections seem to remain rare as several studies report no infection: 2400 mammals, including dogs and cats (Groen et al., 1995); various mammal species (Jay et al., 1997). However Hantaan (HTNV) serotype was detected in a bird (Dzagurova et al., 1995).

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The presence of hantavirus in dogs or cats has never been reported in Belgium. This study first supposed that antibodies against hantavirus were detectable in dog and cat sera in Belgium. In the case of a positive result, this work would test two subsequent hypotheses. Firstly it is known that human PUUV-seroprevalence is linked to the quantity of forests, a good indicator of bank vole abundance, the PUUV reservoir (Heyman et al., 2002). Will the geographical pattern of hantavirus in dogs and cats be the same than in humans? We therefore first hypothesised that a higher hantavirus seroprevalence would be observed in pets from forested southern Belgium. Secondly, in bank voles, hantavirus presence increases with the age of the rodents (Escutenaire et al., 2000a). Will this age effect observed in companion animals? We hypothesised higher seroprevalence in older pets.

2. Materials and methods

We retrospectively tested 534 pet sera (410 dogs and 124 cats), initially sent to private laboratories for routine veterinary analyses. We favoured samples from two regions isolated from each other: north-west Belgium (hereafter called northern Belgium), known to harbour few cases of human hantavirus infections, and south-east Belgium (called southern Belgium), location of numerous human hantavirus infections. For 360 dogs and 120 cats, the owner address, pet's age and/or gender were available. We compared our results to the number of human hantavirus infections in Belgium in 2003 (data from the Scientific Institute of Public Health: www.iph.fgov.be/epidemio/labo).

We analysed the sera using an IgG PUUV and HTNV enzyme-linked immunosorbent assay (ELISA: Progen, Heidelberg, Germany). Samples that proved to be positive as well as an equal number of randomised samples that tested negative were tested again using an immuno-fluorescent assay (IFA-IgG) directed against various hantaviruses including PUUV (Euroimmun, Lubeck, Germany). We followed the manufacturer's instructions. The sensitivity and the specificity of both tests are 97–99.5%. It should be noted that serological testing does not enable the definite identification of the infecting hantaviral serotype due to cross-reactions between serotypes.

As, to our knowledge, positive and negative control sera for dogs and cats are not available, the cut-off (CO) for ELISA was calculated as in previous studies (e.g. Heyman et al., 2002: average optical density (OD) of 10 known negative sera plus 3 times their standard deviation. We compared series by calculating the ratio: observed OD/CO. A sample was considered as positive if this ratio was at least to 1.5. Ratios between 1.0 and 1.5 were considered "grey zone" values. This avoided overestimating the prevalence.

All statistical tests were two-tailed with a level of significance at 0.05 except when indicated otherwise (e.g. age effect on seroprevalence because of our initial hypothesis). We used non-parametric statistics when normality was not expected.

3. Results

We detected hantavirus antibodies in both regions with a higher seroprevalence in cats than in dogs (16.9% vs. 4.9%, Mann–Whitney U test, $N = 534$, $P = 0.0012$, Fig. 1 and Table 1). In IFA, all samples showed a higher titre for PUUV (cross-reacting with Tula, TULV) than for Seoul (SEOV). For both species, the average ELISA ratios were higher in more forested south than in northern Belgium (Mann–Whitney U test: dogs, 0.838 vs. 0.581, $N = 410$, $P = 0.000$; cats, 1.005 vs. 0.899, $N = 124$, $P = 0.010$). Moreover more dogs were infected in southern Belgium than in northern Belgium (10.5% vs. 3.0%, Mann–Whitney U test, $N = 410$, $P = 0.002$, Table 1). This geographical difference in dog seroprevalence is consistent with the number of human cases reported in 2003 (North: 1.9 vs. South: 30.0 individuals per million). Moreover the cat prevalence was influenced by the forest cover in each studied administrative localities (http://www.statbel.fgov.be). Indeed, in the South, PUUV sero-positive cats were found in more densely forested localities than PUUV sero-negative ones (30.5% vs. 15.2%, one-tailed Mann–Whitney U test, $N = 52$, $P = 0.033$). We did not observe such a relationship for dogs.

The mean pets' age was 8.6 years for dogs (SD = 3.7, $N = 105$) and 7.7 years for cats (SD = 5.2, $N = 90$, Table 1). The dogs' age was not linked to the serological status towards hantavirus (one-tailed two-sample T test, $N = 105$, $P = 0.19$). Older cat's showed an almost-significant tendency of higher seroprevalence ($N = 90$, $P = 0.065$, Table 1). Finally, in both species, we observed a non-significant tendency to have higher seroprevalence in females than in males (Table 1).

4. Discussion and conclusions

This study showed that domestic carnivores are susceptible to hantavirus infection in Belgium. This is consistent with the airborne infection route and the pets' behaviour. According to our test results, the observed hantavirus probably correspond to the Puumala serotype. However Tula and Seoul serotypes cannot be ruled out. Indeed only neutralization tests are able to clearly identify the causal serotype. Neutralization tests however are difficult; they require a BSL3 environment and an extensive hantavirus library in culture. Only a few labs worldwide are able to perform such tests and for this study we decided not to seek identification as in Belgium PUUV, TULV and SEOV circulate (Heyman et al., 2002).

It is not known whether carnivores develop any hantavirus disease but low infection rates in dogs and cats suggest dead-end infections, as with humans. In humans, hantaviral RNA is only present during the few days of the acute phase of the disease (Pettersson et al., 2008). We can hypothesise that, as with humans, carnivores neutralise hantaviruses shortly after infection. This very short viraemia hampers in most cases virus isolation from the serum.

It is important to mention that our study involves a bias as all tested pets were sampled in veterinary laboratories for a health problem or a routine health check. This absence of randomised sampling is difficult to avoid and is typical in such epidemiological studies. We can indeed compare our results to those of previous works. We observed a seroprevalence of 4.9% in dogs and 16.9% in cats. This is similar to what was previously reported for dogs in America (4.7%, Malecki et al., 1998 but our results concerning cats were higher than any previous observations (2.9–9.6%, China, Austria and Canada: (Xu et al., 1987; Nowotny, 1994; Leighton et al., 2001). The difference in cats cannot be attributed to our method, as we defined a high cut-off for being positive. The high cat seroprevalence could be partly explained by a bank vole demographic peak in 2005, accompanied by a steep increase of the number of human cases (Heyman et al., 2007). Finally, it is interesting to note that, in one study, 23% of chronically ill cats in the UK were found to be hantavirus-seropositive (Bennett et al., 1990).

Nevertheless, the higher dog PUUV-seroprevalence observed in southern than in northern Belgium allowed us to demonstrate that the same geographical difference exists in dogs and in humans. The higher hantavirus infection rate of cats compared to dogs can be linked to the cats' specialisation in catching small rodents (30% of domestic cat preys (Woods et al., 2003). This is consistent with the fact that sero-positive cats from southern Belgium are found in more forested environments than sero-negative ones.

Contrary to what is observed in the rodent reservoirs (Escutenaire et al., 2000a), we did not detect an age-dependency