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Short Communications

<u>Title</u>: Wild red foxes (*Vulpes vulpes*) as sentinels of Rodent borne virus (Hantavirus and Lymphocytic Choriomeningitis Virus) infections in the Province of Soria, Northern Spain

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ABSTRACT

Three hundred and fourteen red foxes (*Vulpes vulpes*) from the Province of Soria, Spain are examined for Hantavirus and Lymphocytic Choriomeningitis Virus (LCMV) infection. Immunofluorescence and Western blot assays showed 3.50% to have antibodies to hantaviruses, and immunofluorescence assay showed 2.23% to have antibodies to LCMV. The serologic status of the animals showed no association with sex or age. The results show that foxes can be good sentinels of hantavirus and LCMV infection.

KEY WORDS: Animal sentinel, Epidemiology, Hantaviruses, Lymphocytic choriomeningitis virus

Though hantaviruses an arenaviruses have been around for many decades, recently have proposed Tagliapietra et al (2018) in a study carried on in north Italy rodent-borne viral zoonosis -hantaviruses and arenaviruses- are an emerging public health threat and epidemiologic vigilance requires attention by health authorities. Hantaviruses are nearly worldwide rodent-borne pathogens infecting humans and others animals mainly through inhalation of aerosols contaminated with rodent excreta. Hantaviruses are zoonotic viruses harbored most notably by rodents but also bats, moles and shrews as Sabino- Santos et al., (2015) has shown in Brazil.

In a study performed in Belgium (Escutenaire et al., 2000) antibodies against Puumala hantavirus (PUUV) were mainly detected in the bank vole (*Myodes glareolus*) but also in the wood mouse (*Apodemus sylvaticus*) and in the red fox (*Vulpes vulpes*). So, although the natural reservoir of PUUV in Europe is the bank vole it may be in other animals.

Although rodents are important reservoirs for a large number of zoonotic pathogens arenavirus antibodies are a common finding in numerous rodent species (Kallio-Kokko et al., 2006). One arenavirus lymphocytic choriomeningitis virus (LCMV) is often detected in mice and may also be detected in hamsters. In humans LCMV infection can cause meningitis, multisystemic failure in transplant recipients and severe developmental defects in the fetus when infection occurs during pregnancy.

Contact between certain wild and domestic animals indirectly increases contact between wild animals and humans and, therefore, the likelihood of pathogen transmission. Foxes often live in peri-urban and agricultural areas and adapt well to the presence of domestic animals and humans (Fishman 2004). So, although it is generally accepted that foxes do not serve as viral reservoirs (Malecki 1998), foxes may become infected while feeding on rodents and are considered good indicators of vial circulation in their feeding ranges and can be used as sentinels for human infection.

The literature contains no information on whether the foxes of central Spain are infected by rodent-borne hantaviruses and arenaviruses, so our objective was to investigate the prevalence of antibody to these viruses in foxes in the Province of Soria (42º08’20”N to 41º04’15”S, and 01º47’45”E to 03º31’45”W). This is the largest serosurvey of wild foxes for hantas/arenas ever published.

The 314 red fox serum samples used in this work were from our group’s frozen (-20ºC) serum collection. The foxes were captured alive (n=290 foxes) or found dead (n=24), usually as road kills or by forestry agents. Specimen collection lasted four years.

153 foxes were male (48.7%) and 161 female (51.3%); 49 (15.60%) were juvenile (i.e., with milk teeth; <1 yr old), 182 (57.96%) were adult (i.e., with permanent, unworn teeth; 1-5 years old), and 83 (26.43%) were old (i.e., with markedly worn teeth; over 5 yr old).

Serum samples were examined for antibodies using an in- house indirect immunofluorescence assay (IFA), following the method of Lledó et al, (2002) for hantaviruses and Lledó et al, (2003) for LCMV. PUUV (strain Cg18/20), SEOV (strain 80/39), and LCMV (strain Armstrong) were used as antigens. These were propagated in VeroE6 cells (ATCC CRL 1586) and L-929 cells (ATCC-CCL 1) for hantaviruses and LCMV respectively, and fixed on spot slides. The fluorescein-labeled conjugate used was a rabbit anti-dog IgG serum (Sigma, St. Louis, Missouri, USA), diluted 1/128 in phosphate-buffered saline (PBS) containing Evan blue. Sera showing a typical pattern of fluorescence at titers ≥1/16 were considered positive. Spots of uninfected Vero E6 cells and L-929 cells were used as negative controls.

Serum samples with IgG titers ≥1:16 (as determined by IFA) were analyzed by Western blot to detect anti-hantavirus IgG antibodies. Blotting for hantaviruses was performed exactly as described by Hjelle and others (1997). The recombinant N proteins of PUUV and SEOV, were expressed using the pET23b vector (Novagen, Madison, Wisconsin, USA) in *Escherichia coli* BL21 (Novagen) and purified in a metal chelation column using a C-terminal polyhistidine moiety.

Positive and negative control sera (provided by European Network for Diagnostics of Imported Viral Diseases) were also examined.

Differences in antibody prevalences between sexes and age classes in two-way contingency tables were analyzed using $\chi 2$ or Fisher's exact tests. Significance was set at $P < 0.05$.

Permission to take and study these animal samples was obtained from the regional government of Castilla y León in compliance with current legislation (Protocol 06.01.017.006). Sampling followed the ethical guidelines of the Committee on Animal Experimentation of the University of Alcalá de Henares (Protocol CEI 2011034).

Eleven foxes had antibodies against hantaviruses. Antibody prevalences were

4.96% (8/161) in female foxes and 1.96% (3/153) in males. Eight adult animals (4.39% [8/182]) were antibody positive, one was juvenile (2.04% [1/49]), and two were old (2.40% [2/83]). Positive serum sample titers ranged from /32 to /512 (Table 1).

Seven foxes had antibodies to LCMV (prevalence 2.23% [7/314]); five (3.10% [5/161]) were females and two (1.30% [2/153]) were males; five (2.74% [5/182]) were adult, and two (2.40% [2/83]) were old. Positive serum sample titers ranged from /32 to /64 (Table 1).

We found no statistically significant differences in the prevalences of any infection with respect to age or sex.

Although much is known about rodent reservoirs, little is known regarding other wild animals as hantavirus and LCMV reservoirs. For hantaviruses, numerous recent studies have demonstrated the potential of bats, shrews and moles as potential reservoirs. However, some other previous studies with other carnivores as coyotes from New Mexico and northeastern Arizona they were shown not act as reservoirs (Malecki et al., 1998) but perhaps they quickly develop antibodies, clear the infection, and do not pass it on. They could probably dead-end hosts just like cats and humans.

In Europe there are few studies on Hantavirus infection in mammalian predators of rodents, and most have focused on the cat as domestic predator (Nowotny et al., 1994). In Belgium it was detected in pets' sera of dogs and cat's hantavirus antibodies (Dobly et al., 2012) and authors were found a significant higher seroprevalence in cats than in dogs in southern Belgium than in northern Belgium related to the highly forested southern Belgium that harbouring more rodents than in northern Belgium, stablishing a possible one relation with the ecological variations of hantavirus. In the case of wild predators such as the fox a previous study showed an antibody prevalence of 2.4% (Escutenaire et al., 2000); in our study we found a prevalence (3.5%).

Referent to LCMV is maybe also important the role of pets (Reperant et al., 2016) and is needed in-depth analysis of the risk of companion animals as sources of viruses for human because is one of the causing agents of aseptic meningitis in Spain (De Ory et al., 2009). So, in southern Spain the prevalence of LCMV in meningitis patients was low but represented 2.9% of all pathogens detected being LCMV proposed a noteworthy agent of neurologic illness in immunocompetent persons (Pérez-Ruiz et al., 2012).

Related to LCMV, its known the role of rodents in the transmission. Our results have showed for the first-time red foxes LCMV with a prevalence of about 2.23%, initially low, but is also important to know what happens with other animals, predator mammals of these rodents than can act in the maintenance of the viruses in the nature.

Red foxes are now common in many cities worldwide and in the recent years have colonized urban areas (Plumer et al., 2014), seems to use the presence of rodents (Baker et al., 2000) and are available to use anthropogenic food sources (Mueller et al., 2018).

Perhaps the presence of other reservoirs (wild and domestics) than rodents emerging different epidemiological cycles in nature involving rodents and carnivores as red foxes in the case of Europe and involving bats and shrews and some other carnivore not yet identified in America. In this short communication we have detected the presence of antibodies but the role as reservoir or its implication in the transmission cycle of the virus, will be something that requires further epidemiological studies in which could be detected live virus in the subjects studied.

Author Disclosure Statement

No competing financial interests exist.

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Table 1- Serum dilutions antibodies (IgG) against the studied microorganisms organisms

Positives	LCMV	PUU	Seoul
1	1/32		
2	1/32		
3	1/64		
4	1/32		
5	1/64		
6	1/32		
7	1/32		
8		1/64	1/128
9		1/64	1/128
10		1/128	1/64
11		1/32	1/128
12		1/32	1/128
13		1/256	1/256
14		1/128	1/128
15		1/128	1/32
16		1/128	1/512
17		ND	1/128
18		1/256	1/64

ND: Not determined