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Seroprevalence of hantaviruses and *Leptospira* in muskrat and coypu trappers in the Netherlands, 2016

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ABSTRACT

Aims: Seoul orthohantavirus (SEOV) and *Leptospira* spp. are zoonotic pathogens with rats as main reservoir. Recently, the presence of SEOV in brown rats was reported in one region in the Netherlands. Brown rats are a frequent bycatch in traps placed to catch muskrats (*Ondatra zibethicus*) and coypus (*Myocastor coypus*), and thus are a potential health risk for trappers. It was our aim to determine the seroprevalence of orthohantavirus, specifically SEOV, and *Leptospira* spp in Dutch trappers. **Methods and results:** Participating trappers provided serum samples and completed an online questionnaire. The serum was tested for the presence of antibodies against six orthohantaviruses and eight *Leptospira* serovars. Two hundred-sixty trappers completed the online questionnaire (65%), and 246 (61%) and 162 (40%) serum samples were tested for relevant orthohantaviruses and *Leptospira* spp., respectively. The seroprevalence of Puumala orthohantavirus in Dutch trappers was 0.4% (95% CI: 0.1–2.3%). None of the participants tested positive for SEOV. The seroprevalence of leptospirosis was 1.2% (95% CI: 0.3–4.4%), although *Leptospira* spp. are present in brown rats in the Netherlands.**Significance of study:** The results indicate that the infections with orthohantaviruses and leptospires is low for muskrat and coypu trappers.

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Introduction

Rats are important carriers of zoonotic pathogens [1] and handling of these animal species may increase the risk of transmission of zoonoses. In the Netherlands, brown rats (*Rattus norvegicus*) are known to be a reservoir for both Seoul orthohantavirus (SEOV) and *Leptospira* spp [2].

Cross-sectional studies testing brown rats for leptospires in the period 2011–2015 showed positivity rates ranging from 33% to 57% per area, tested by real-time PCR and/or culture [unpublished data].

In addition, in February 2015, Verner-Carlsson et al. [2] reported the presence of SEOV in three of 16 brown rats captured in an area in the east of the Netherlands in 2013. Previous cross-sectional monitoring studies in 150 brown rats in three different Dutch areas performed from 2011 to 2015 found no evidence of SEOV [unpublished data].

The majority of symptoms associated with infections with orthohantavirus and leptospirosis are aspecific, making them difficult to identify based on clinical presentation only. Infections with orthohantaviruses circulating in Europe, such as SEOV and Puumala orthohantavirus (PUUV), range from asymptomatic to lethal [3,4]. Overall, SEOV causes a more severe clinical picture than PUUV [5]. Symptoms associated with infection are fever, headache, backache, and gastrointestinal symptoms. Ocular problems, renal and liver impairment, and haemorrhage can be seen in severe cases. Leptospirosis also manifests mostly subclinically or as a mild, self-limited illness [6,7]. Most common symptoms are fever, (severe) headache, muscle pain, cough, and gastrointestinal symptoms. In severe cases of leptospirosis, multiple organs dysfunction including kidneys, liver, lungs, and brain can occur. In the Netherlands, an increase in human leptospirosis has been seen in recent years [8,9] and while over 100 cases of PUUV infections have been reported since 2008, no SEOV infections related to wild rats have been diagnosed in humans to date.

The finding of SEOV in wild brown rats [2] and the common frequent *Leptospira* spp infection of brown rats, raised questions about potential health risks for muskrat trappers as brown rats are a frequent bycatch in traps placed to catch muskrats and coypus. Therefore, a seroprevalence study was designed, with the aim to determine the seroprevalence of orthohantaviruses, and specifically SEOV, and *Leptospira* spp. in Dutch muskrat and coypu trappers as a measure for potential infection.

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Materials and methods

Study population, data collection and data analysis

Muskrat and coypu trappers working at the regional water authorities were invited to participate in the study consisting of providing a serum sample through a finger prick and completing an online questionnaire. All muskrat and coypu trappers of all regional districts received an email, via their work e-mail address, with information about the study and a link to the online questionnaire. Five regional meetings covering all districts and organized in June 2016 were used to further introducing and explaining the study. At the meeting, written informed consent was obtained and a blood sample was collected in a tube. The questionnaire contained questions about demography, health, symptoms and complaints in the previous year, occupational exposures to water and animals, including availability and use of personal protective equipment, and possible non-occupational exposures. Use of various personal protective measures was asked with the answer options: almost always, often, sometimes, and never. In the analyses almost always and often were combined into 'regularly'. The data was analysed using SAS 9.4 (SAS Institute Inc., USA). Due to the small number of positive serum samples, analysis was restricted to descriptive analyses. Seroprevalence was calculated as percentage with 95% confidence intervals (95% CI). The Medical Research Ethics Committee of the UMC Utrecht approved the study design (nr. 16–095).

Serology

The blood samples were processed and serum was stored at −20°C at the National Institute for Public Health and the Environment (RIVM) in Bilthoven until further use. All sera were screened for orthohantavirus-specific IgG at serum dilution of 1:100 using a commercially available ELISA (Hantavirus IgG Dx Select; Focus Diagnostics, Cypress, CA, USA) that can detect antibodies against the most clinically relevant orthohantaviruses in Europe according to the manufacturer's instructions. Confirmatory testing was done on all ELISA positive samples using a commercially available immunofluorescence assay (IFA) (Anti-Hantavirus IIFT (IgG); EuroImmun, Germany) to detect IgG against six orthohantaviruses (Hantaanvirus, PUUV, SEOV, Sin Nombre virus, Dobrova virus, and Saaremaa virus) according to the manufacturer's instructions, with 2-fold dilutions from 1:32 to 1:2048.

All available sera, after testing for antibodies against orthohantavirus, were sent to the National Reference Laboratory for Leptospirosis (NRL) in Amsterdam to be screened for *Leptospira*-specific agglutinating antibodies using the microscopic agglutination test (MAT) with final serum dilutions of 1:20, 1:40, 1:80, 1:160 and 1:320. The samples were tested for eight relevant serovars (serogroup/serovar). The pathogenic serovars Australis/Bratislava, Ballum/Ballum, Grippotyphosa/Grippotyphosa type Duyster, Icterohaemorrhagiae/Copenhageni, Icterohaemorrhagiae/Icterohaemorrhagiae, Javanica/Poi, and Sejroe/Hardjo were included since these were the most commonly found serovars in the Netherlands [8,10]. The saprophytic serovar Semaranga/Patoc was added because this can cross react with human antibodies generated by a number of pathogenic serovars [11].

Results

A total of 402 muskrat and coypu trappers were invited to participate in the study of whom 260 persons completed the online questionnaire (65%). In addition, blood samples were taken from 246 persons (61%), which were tested for IgG against six orthohantaviruses. Sufficient serum was obtained from 162 participants (40%) to test for antibodies against *Leptospira* as well. Of 212 participants (53%), we had a questionnaire and a serum sample.

Questionnaire

The median age of the participants completing the questionnaire was 50 years (range: 22–65 year) and almost all were male (257/260; 99%). The participants worked a median of 15 years (range: <1–42 years) for the water authorities, and all 22 regional water authorities were represented. The majority (89%) had at least weekly contact with surface water. Table 1 shows the number of trappers who came in contact with muskrats (248; 95%), coypus (48; 18%) and brown rats (232; 89%). When trappers came in contact with

Table 1. Number of catches of, methods of catching of and weekly contact with muskrats, coypus and brown rats.

	Muskrat	Coypu	Brown rat
Participants with contact	248 (95%)	48 (18%)	232 (89%)
Mean number of catches per month:			
* dead rats	17		4
* living rats	2	5	2
Method of catching:			
* drown trap	32%	-	33%
* trap	59%	-	49%
* cage – captured alive, shot	6%	89%	15%
* captured alive, stricken dead	3%	11%	3%
Percentage of participants with weekly contact with the concerned rats:			
Total	76%	46%	42%
* dead rats	75%		36%
* living rats	12%	44%	14%
* killing of rats	25%	38%	15%
* bite incident rats	1%	4%	<1%
* contact eyes/ mucosa with rat urine or blood	13%	21%	8%

the particular type of rat, this was in 76% (muskrats), 46% (coypus) and 42% (brown rats) at least weekly. Muskrat and coypu trappers had less often contact with mice: 46% of the trappers reported contact with mice, and only 12% came in contact with mice at least weekly. The availability of personal protective equipment was overall rated as good. The majority of the participants wore gloves regularly (88–94%, depending on season with highest compliance in winter) and waders (94%). Protection of the eyes and change of clothes when wet is done regularly by half the participants. Masks to protect nose and mouth were less often used regularly (11%).

Seroprevalence

Based on the initial screening of sera by ELISA, 15 of the 246 serum samples showed serological reactivity for orthohantavirus, of which 11 were considered positive and 4 were considered borderline based on the cut-off determined by the manufacturer. Out of these 15 sera, only one serum could be confirmed in the IFA. This serum tested positive in the IFA with the highest titre of 1:2048 for PUUV and Sin Nombre virus IgG. Sin Nombre virus is known to cross-react with serum from PUUV infected individuals. Additionally, a four-fold lower cross-reactivity was observed with the other orthohantaviruses, including SEOV, Dobrova virus, Hantaanvirus and Saaremaa virus. The concerning participant works at the water authority for over a decade, and did not report symptoms compatible with a orthohantavirus infection in the previous year. The seroprevalence of orthohantavirus, specifically PUUV in this study is 0.4% (95% CI: 0.1–2.3%). No evidence of SEOV or other orthohantaviruses specific IgG was detected in any of the participants.

Two of 162 serum samples tested weak positive for *Leptospira* (titres 1:20 and 1:40), in particular serovar Bratislava from serogroup Australis, corresponding to a seroprevalence of 1.2% (95% CI: 0.3–4.4%). Both participants work at the water authority for over a decade, and both had an episode of general symptoms without fever (flu-like symptoms including gastro-intestinal complaints, and a combination of headache, muscle ache and joint pain), in the previous year. A striking feature of both participants was that both reported to (almost) never wear gloves between spring and fall, and only sometimes to (almost) never in winter, as was reported by merely 2% of the participants.

Discussion

Muskrat and coypu trappers have frequent contact with surface water and brown rats. When trapping muskrats and coypus, other animals are also captured. Brown rats are a frequent bycatch in traps comprising 34% of those animals in 2015 [12]. Brown rats can be a reservoir for important zoonotic pathogens like SEOV and leptospires, and when infected they can excrete pathogens and contaminate their environment. The risk of infection is determined by the level of infection in the reservoir, contamination of the environment, conditions affecting virus viability in the environment, and the frequency and intensity of exposure.

The results of this study in combination with data on infection rates of brown rats suggest that the risk of contracting a SEOV infection is very low. In a parallel study of brown rats, captured in 2015 in the same district as the three confirmed positive rats from 2013, none of 53 brown rats tested positive for SEOV [unpublished data]. Also, of the 97 brown rats tested in the period 2011–2015 in three other areas, all were SEOV negative [unpublished data]. None of the participants showed serological evidence of infection with SEOV.

In an earlier study in Dutch muskrat trappers, all 67 participants tested negative for orthohantavirus [13]. The present seroprevalence of PUUV (0.4%; 95%CI 0.1–2.3%) is comparable to the seroprevalence estimated in the Dutch (1.7%; 95%CI 1.3–2.3%), Belgian (1.5%) and German (1–3%) general population [14–16]. The only orthohantavirus found in our study was identified as PUUV which is believed to cause the majority of human orthohantavirus infections in the Netherlands [14], and is associated with bank voles (*Myodes glareolus*). Contact with mice was less often reported by muskrat and coypu trappers than contact with rats, and could explain a similar seroprevalence as found in the general population. The IFA enables highly sensitive and specific serological diagnosis of orthohantavirus infections and can be used to differentiate PUUV infection from infections with SEOV [17]. Interestingly, 14 participants tested positive for orthohantavirus specific IgG using a pan-orthohanavirus assay, however these could not be confirmed using an immunofluorescent assay.

In four cross-sectional studies performed at different locations in the Netherlands, *Leptospira* spp. infection in brown rats ranged from 33 to 57% [unpublished data]. The seroprevalence of leptospirosis in muskrat and coypu trappers was 1.2% (95%CI 0.3–4.4%) in the present study. In an earlier study, three of 67 muskrat trappers (4%) tested positive [13]. One explanation of the low seroprevalence in relation to the high level of positive rats could be the use of personal protective equipment, especially the gloves and the waders. In addition, the seroprevalence represents recent rather than lifelong exposure, since antibody titres to leptospires do not remain detectable lifelong, but may vary from months to years [6,18].

Asking whether personal protective measures are taken could be prone to social desirable answering. Nevertheless, as the easy to implement measures (i.e. gloves) were rated high in use and the more difficult or unpleasant ones (i.e. nose-mouth masks) were only used by a minority, overestimation of use is expected to be minimal.

For practical and logistic reasons, blood was taken with a finger prick. While lowering the threshold for participation, a drawback of this method was the limited amount of blood available. It resulted in exclusion of some participants for testing for evidence of previous infection with leptospires, leading to a broader confidence interval.

In conclusion, the results indicate that infection via work-related exposure to orthohantavirus, and more specifically SEOV, is low for muskrat and coypu trappers. This is probably due to the low levels of infection in the reservoir. Although leptospires in brown rats are rather common, work-related infections were rare. The use of personal protective equipment may have an important role in preventing contact with leptospires.

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Disclosure statement

No potential conflict of interest was reported by the authors.

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