WILDLIFE

Meningitis and orchitis in a hare (*Lepus europaeus*) infected with *Francisella tularensis*

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SUMMARY

In southern Belgium, a brown hare (*Lepus europaeus*) was found moribund and killed for ethical reason. The animal was transmitted for postmortem examination. Major histopathological findings consisted of multifocal subacute necrotising meningitis and multifocal subacute necrotising orchitis. Infection with *Francisella tularensis* was confirmed by both bacteriological isolation and detection by real-time PCR. Further, subtyping of *F tularensis* colonies stated that it was *F tularensis* subspecies *holarctica* biovar *I*. It is the first case of tularaemia detected in wildlife in Belgium since 2003. The event pushed health professionals to communicate with hunters and other groups with outdoor activities about the *Francisella* risk and the ways to take care of it, such as wearing gloves to handle found-dead or hunted hares and taking protective measures against tick bites.

BACKGROUND

Tularaemia is a zoonotic disease caused by *Francisella tularensis*. Currently, 4 subspecies are described and two of them are known to pose a threat to human health: *F tularensis* subspecies *tularensis* and *F tularensis* subspecies *holarctica*. The first is the most virulent subspecies and occurs in North America while the second is mainly present in Europe and Asia, and to lesser extent, in North America (Foley and Nieto 2010). However, some strains of *F tularensis* subspecies *tularensis* were isolated from fleas and mites parasiting on small terrestrial mammals, collected in Slovakia (Gurycova 1998). The European brown hare (*Lepus europaeus*) plays an important role in the epidemiology of tularaemia as amplifying host and reservoir in western Europe (Gyuranecz and others 2010, Decors and others 2011, Rijks and others 2013) and the species is moderately susceptible to the disease compared with the mountain hare (*Lepus timidus*) (Morner and others 1988). The main gross pathological feature of the disease in the brown hare is the presence of numerous necrotic foci in organs. The most often affected tissues in brown hares in Hungary were lungs, pericardia and kidneys (Gyuranecz and others 2010). Chronic pattern with poor body condition is also frequently reported in hares (Morner and others 1988, Gyuranecz and others 2010). A recent study performed in Switzerland on *F tularensis* subspecies *holarctica* naturally infected brown hares demonstrated that lesions were different according to the phylogenetic cluster involved; splenitis and hepatitis were associated with one cluster while polyserositis affecting pleura, pericardium and kidney were associated with the other (Origgi and Pilo 2016).

Human infection may result from inhalation of infective aerosols, skin and conjunctivae contact with or ingestion of infected hosts or water, and tick bites (Mailles and Vaillant 2014).

CASE PRESENTATION

In September 2011, in southern Belgium (province of Liege), a young male brown hare was found moribund by a hunter and killed for ethical reason. The animal was transmitted to the Surveillance Network of Wildlife Diseases for postmortem examination (Linden and others 2011). At postmortem examination, numerous whitish foci, less than 1 mm of diameter, were recorded bilaterally on the surface of the testis, the epididymis and the deferent duct (Fig 1). The mesenteric lymph nodes were slightly enlarged. The spleen was doubled in volume, with a firm consistence. No gross lesion was apparent at examination of the brain. The animal showed a poor body condition with muscle atrophy and absence of fat deposits on the organs. Presence of a milk-like fluid was noticed in the stomach. Samples from various organs were examined by histopathology. Lung and kidney showed congestion. A mild subacute multifocal necrotising inflammation was observed in the liver. Major findings were reported in the CNS (cortex, midbrain, cerebellum and medulla oblongata) with multifocal subacute necrotising meningitis and local congestion (Fig 2), and with several foci of subacute necrotising inflammation at the periphery of the testis, in the tunica albuginea. Mesenteric lymph nodes showed no abnormal microscopic findings.

FIG 1: Gross postmortem view showing the testis covered by numerous whitish foci

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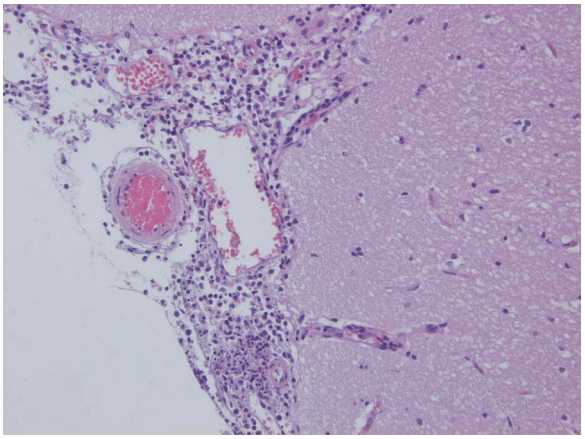

FIG 2: Histological view of brain (cortex of frontal lobe) showing subacute necrotising meningitis. Haematoxylin and eosin ×20

Infection with *F tularensis* was confirmed by both conventional bacteriological isolation and detection by real-time PCR (Versage and others 2003) performed in parallel on spleen, liver and testis. All procedures were performed under biosafety level 3 conditions. Tissue samples were cultured on Chocolate agar supplemented with cystein and on Francis agar medium. The putative colonies isolated by culture were identified using phenotypical characters (Gram staining, oxidase and catalase tests), and the species identification was confirmed by specific nested PCR targeting *tul4* gene (Peruchon and others 2006). Further, subtyping of *F tularensis* colonies was performed by PCR targeting the genomic region RD1 that allows the differentiation between *F tularensis* subspecies *tularensis* and *F tularensis* subspecies *holarctica* (Broekhuijsen and others 2003). Biovar I identification of isolate was carried out by erythromycin sensitivity test (Kudelina and others 1980). Spleen, liver and testis were PCR-positive but brain tissue was not available for genetic analysis. However, meningitis recently described in *F tularensis* subspecies *holarctica* infected hares (Origgi and Pilo 2016) suggest that subacute necrotising meningitis observed in the present study could be related to tularaemia infection.

DISCUSSION

The last occurrence of tularaemia in Belgium occurred in 2003 with isolation of *F tularensis* subspecies *holarctica* from a dead hare in southern Belgium (province of Namur). In the same year, two human cases of tularaemia were diagnosed by serological tests. The two patients showed clinical signs of the disease (flu-like syndrome with fever, headaches and axillary adenopathy with lymphangitis) and they admitted handling and close contact with a dying hare (Walravens and others 2005). After this event, a surveillance programme was carried out in Wallonia during two years. A total of 30 spleens of hares presenting suggestive lesions were tested (culture and PCR) and all were negative (Walravens and others 2005).

This case is questioning for public health authorities because it is not known whether it is truly sporadic or whether it is the only case from an endemic focus that was detected by the currently low-level surveillance programme. Surveillance for tularaemia in southern Belgium wildlife should be strengthened for clarifying this point. The hunter who discovered this animal did not display fever or expected symptoms after handling the carcase. Unfortunately, he was never serologically tested for tularaemia. The event pushed health professionals to specifically communicate with hunters and other groups with outdoor activities about the *Francisella* risk and the ways to take care of it, such as wearing gloves to handle found-dead or hunted hares and taking protective measures against tick bites.

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REFERENCES

Broekhuijsen M., Larsson P., Johansson A., Bystrom M., Eriksson U., Larsson E., Prior R. G., Sjostedt A., Titball R. W., Forsman M. (2003) Genome-wide DNA microarray analysis of *Francisella tularensis* strains demonstrates extensive genetic conservation within the species but identifies regions that are unique to the highly virulent *F. tularensis* subsp. *tularensis*. *Journal of Clinical Microbiology* 41, 2924–2931

Decors A., Lesage C., Jourdain E., Giraud P., Houbron P., Vanhem P., Madani N. (2011) Outbreak of tularaemia in brown hares (*Lepus europaeus*) in France, January to March 2011. *Euro Surveillance* 16

Foley J. E., Nieto N. C. (2010) Tularemia. *Veterinary Microbiology* 140, 332–338

Gurycova D. (1998) First isolation of *Francisella tularensis* subsp. *tularensis* in Europe. *European Journal of Epidemiology* 14, 797–802

Gyuranecz M., Szeredi L., Makrai L., Fodor L., Meszaros A. R., Szepe B., Fuleki M., Erdelyi K. (2010) Tularemia of European Brown Hare (*Lepus europaeus*): a pathological, histopathological, and immunohistochemical study. *Veterinary Pathology* 47, 958–963

Kudelina R. I., Olsufjev N. G. (1980) Sensitivity to macrolide antibiotics and lincomycin in *Francisella holarctica*. *Journal of Hygiene, Epidemiology, Microbiology, and Immunology* 1, 84–91

Linden A., Wirtgen M., Volpe R., Nahayo A., Pirson J., Paternostre J., Gregoire F. (2011) Surveillance of wildlife diseases in Belgium. *Epidémiologie et Santé Animale* 59–60, 213–215

Mailles A., Vaillant V. (2014) 10 years of surveillance of human tularemia in France. *Euro Surveillance* 19, 45

Morner T., Sandstrom G., Mattsson R., Nilsson P. O. (1988) Infections with *Francisella tularensis* biovar *palaearctica* in hares (*Lepus timidus, Lepus europaeus*) from Sweden. *Journal of Wildlife Diseases* 24, 422–433

Origgi F. C., Pilo P. (2016) *Francisella tularensis* clades B-FTN002-00 and B-I3 are associated with distinct pathology in the European Brown Hare (*Lepus europaeus*). *Veterinary Pathology* Published Online First: 1 Mar 2016. doi:10.1177/0300985816629718

Peruchon S., Henault S., Mendy C., Vaissaire J. (2006) Diagnostic de la tularémie par amplification génique in vitro (PCR). *Bulletin de l'Académie Vétérinaire de France* 159, 165–168

Rijks J., Kik M., Koene M., Engelsma M., van Tulden P., Montizaan M., Oomen T., Spierenburg M., Ijzer J., van der Giessen J., Gröne A., Roest H. (2013) Tularemia in a brown hare (Lepus europaeus) in 2013:first case in the Netherlands in 60 years. *Euro Surveillance* 18, pii 20655

Versage J. L., Severin D. D., Chu M. C., Petersen J. M. (2003) Development of a multitarget real-time TaqMan PCR assay for enhanced detection of *Francisella tularensis* in complex specimens. *Journal of Clinical Microbiology* 41, 5492–5499

Walravens K., Bughin J., Roels S., Sion C., Linden A., Reginster M., Godfroid J., Govaerts M., Gregoire F., Troussart J. M., Saegerman C. (2005) Tularaemia, an emerging zoonosis in Belgium? *First Symposium of the Belgian Wildlife Disease Society*, Brussels

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