International Journal for Parasitology: Parasites and Wildlife 5 (2016) 277–279

Contents lists available at ScienceDirect

International Journal for Parasitology: Parasites and Wildlife

journal homepage: www.elsevier.com/locate/ijppaw

Current opinion

First findings of *Trichinella spiralis* and DNA of *Echinococcus multilocularis* in wild raccoon dogs in the Netherlands

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ARTICLE INFO

Article history:
Received 19 July 2016
Received in revised form
6 September 2016
Accepted 15 September 2016

Keywords:
Raccoon dog
Echinococcus multilocularis
Trichinella spp.
Wildlife
Zoonosis
Public health

ABSTRACT

The recent invasion of the raccoon dog in the Netherlands may be associated with the risk of introduction and spread of zoonotic pathogens. The aim of this study was to assess whether *Echinococcus multilocularis* and *Trichinella* spp. infections are present in Dutch raccoon dogs. Between 2013 and 2014, nine raccoon dogs, mainly road kills, were collected for necropsies. One raccoon dog tested repeatedly positive in the qPCR for *E. multilocularis*. The positive raccoon dog was collected in the province of Flevoland, which is not a known endemic region for *E. multilocularis*. Another raccoon dog tested positive for *Trichinella spiralis* by the digestion of the forelimb musculature and the tongue. *Trichinella spiralis* has not been reported in wildlife since 1998 and thus far was not found in wild carnivores in the Netherlands. It shows that despite the small raccoon dog population that is present in the Netherlands and the limited number of raccoon dogs that were tested, the raccoon dog may play a role in the epidemiology of *E. multilocularis* and *Trichinella* spp. in the Netherlands.

1. Introduction

The raccoon dog (*Nyctereutes procyonoides*) originates from East-Asia and was introduced to European parts of the former Soviet Union between 1929 and 1955 (Lavrov 1971, in (Kauhala and Kowalczyk, 2011)). In the decades after its introduction, it spread rapidly through Europe (Kauhala and Kowalczyk, 2011). The first sightings in the Netherlands date from the 1980's and 1990's. These likely first concerned intentionally released or escaped pet raccoon dogs and later also wild raccoon dogs (overview in (Mulder, 2013)). In 2012, 2013 and 2015, reproduction of wild raccoon dogs in the Netherlands was recorded in the province of Drenthe and it is expected that the population of this opportunistic omnivore will grow to carrying capacity within 15–30 years, colonizing large parts of the Netherlands (Meijer and Klop, 2014).

The spread of the raccoon dog is associated with the risk of introduction and spread of various zoonotic pathogens (Sutor et al., 2014). Two of these pathogens for which the raccoon dog is considered a public health risk in the Netherlands are *Echinococcus multilocularis* and *Trichinella* spp.

Echinococcus multilocularis, also called the fox tapeworm, is a small cestode that can be found in red foxes in the Netherlands in two distinct regions, in the northeast and the very south (van der Giessen et al., 1999). It can cause the disease alveolar echinococcosis, which can be fatal when not treated (Eckert and Deplazes, 2004). The main definitive host is the red fox, but raccoon dogs are also known to be hosts that shed large numbers of infectious eggs in the environment (Kapel et al., 2006).

Raccoon dogs can also be reservoir hosts for the nematode *Trichinella* spp. (Pozio et al., 2009). This may increase transmission risk to other wildlife, e.g. wild boars and red foxes, or via rodents to domesticated pigs. In the German region of Mecklenburg–Western Pomerania, the regional increase of *Trichinella* spp. that was seen in wild boar since 2005 is suggested to be due to the concurrent increase of the raccoon dog population in that area (Pannwitz et al.,

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http://dx.doi.org/10.1016/j.ijppaw.2016.09.001

2010).

The establishment of the raccoon dog in the Netherlands and the likely increase of its population, raise concerns about their role in the epidemiology of zoonotic pathogens. To gain insight in the role of the raccoon dog in the epidemiology of *E. multilocularis* and *Trichinella* spp. in the Netherlands, raccoon dogs were collected in 2013 and 2014 and studied for these two pathogens. This paper reports on the findings of the necropsies and the diagnostics performed on the nine raccoon dogs collected and the potential public health consequences.

2. Material and methods

Raccoon dogs, whether found dead or shot, were collected in 2013 and 2014. Animals were kept at −80 °C for at least one week, to kill any potential infective eggs of *E. multilocularis*. Subsequently, necropsies were performed and the small intestines and colon contents were collected for *E. multilocularis* diagnostics and the muscles of the front leg (including Musculus carpi ulnaris) and the tongue for *Trichinella* spp. diagnostics.

For the detection of *E. multilocularis*, the sedimentation counting technique (SCT) was used, described by the WHO (Eckert et al., 2001) using the modifications by Hofer et al. (Hofer et al., 2000). Additionally, colon contents were tested using an in-house magnetic capture DNA extraction followed by qPCR (Maas et al., 2014).

For the detection of *Trichinella* spp., the artificial digestion method was used, as described by Franssen et al. (2014a). Of the tongue muscles, 5–10 g were used and of the muscles of the front legs, 20–35 g was used per animal. Digests were performed in 1 L pepsin-HCl digest, using the muscles of the front legs and the tongue muscle. Meat digests were analysed microscopically and isolated *Trichinella* spp. larvae were identified by PCR on the 5S intergenic spacer region and sequencing (Franssen et al., 2015).

3. Results and discussion

Nine raccoon dogs were collected during the study. Seven raccoon dogs were traffic victims, one was shot and one had an unknown cause of death. Six animals were male, and five animals were adult. All animals were in a good condition. They were sent in from the northeastern part of the Netherlands and the province of Flevoland (see Fig. 1).

No raccoon dogs were positive for *E. multilocularis* using the sedimentation counting technique. This could be due to large quantities of sand in the intestines and floating debris, which complicated the sedimentation, or could be due to autolytic samples, also described for red foxes (Isaksson et al., 2014). Using the magnetic capture DNA extraction followed by qPCR using colon contents, there was one raccoon dog that tested repeatedly positive in the qPCR for *E. multilocularis*, with Cp values 35.5 and 36.7 (both 100× diluted). This animal was an adult male, a traffic victim, and was found in Southwest Flevoland (see Fig. 1, green star). This area is currently regarded as an area free from *E. multilocularis*, as previous studies in red foxes were negative in this region. However, the endemic area of *E. multilocularis* may have increased. In 2011, one *E. multilocularis* positive fox from the province of Overijssel was detected during a parasitological survey of red foxes (Franssen et al., 2014b). Previously, it has been calculated that *E. multilocularis* will spread with 3 km/year in the northern part of the Netherlands (Takumi and Giessen, 2009), and with 2.7 km/year in the southern part in northward direction (Takumi et al., 2008). This however, cannot explain the current finding, as the distance between previous findings and the current finding, is larger than predicted. This may be due to the increasing raccoon dog population in the Netherlands, resulting in an increased spread, as raccoon dogs have a high dispersal rate and dispersal distance, reaching up to 300 km in a year or 145 km in a straight line in a couple of months (Kauhala and Kowalczyk, 2011). Whether this single finding indicates an increase of the endemic area up to Flevoland, or that it is a coincidental finding of an animal that roamed a large distance from an endemic area, is unknown. The contribution of the raccoon dog to the environmental contamination with *E. multilocularis* under natural conditions is not known yet. Reported (calculated) prevalences of *E. multilocularis* in raccoon dogs in European countries range from 6% to 12% for the eastern region of Brandenburg (Germany) (Schwarz et al., 2011), to 8.2% in Latvia (Bružinskaitė-Schmidhalter et al., 2012). It needs to be taken into account that raccoon dogs use latrines for their feces, which may result in only a few specific places with a high contamination, whereas foxes spread their feces over their whole territory.

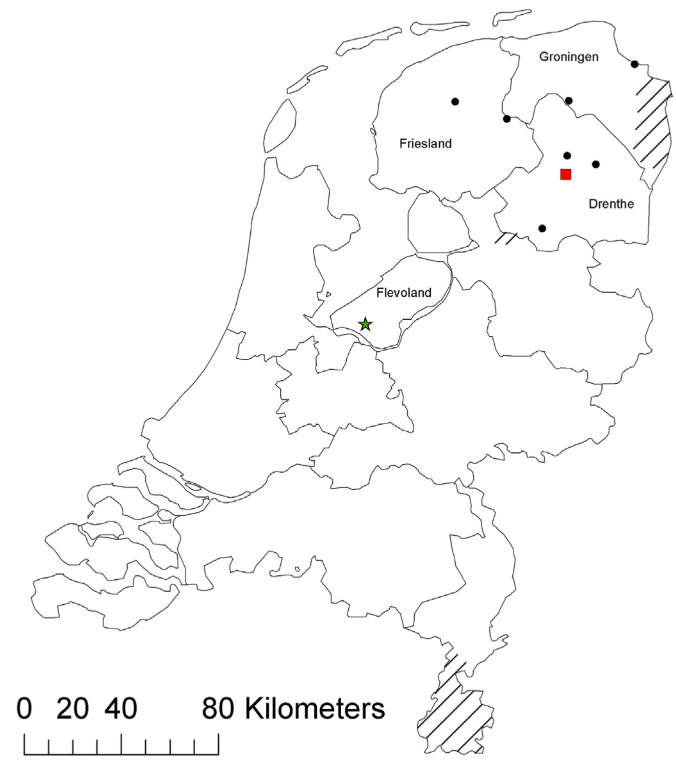

Fig. 1. Finding locations of the raccoon dogs in the Netherlands. Finding locations are indicated with black dots. The finding location of the raccoon dog positive for *E. multilocularis* is indicated with a green star, the finding location of the raccoon dog positive for *T. spiralis* is indicated with a red square. Names of the relevant provinces are shown on the map. The marked areas represent the areas in which red foxes positive for *Echinococcus multilocularis* have been detected in previous studies.

One raccoon dog tested positive for *Trichinella* spp. by the artificial digestion method of the forelimb musculature (89.3 larvae per gram) and the tongue (32.4 larvae per gram). Subsequent molecular identification showed it was *Trichinella spiralis*. This adult male raccoon dog was found in the province Drenthe (see Fig. 1, red square). In the Netherlands, so far only *T. britovi* was found in Dutch wild carnivores (van der Giessen et al., 1998; Pozio et al., 2009). The finding of *T. spiralis* in a raccoon dog is therefore remarkable. *T. spiralis* is one of the most pathogenic *Trichinella* species for humans, and has not been reported in wildlife in the Netherlands after the finding of 2/11 positive wild boar from Limburg in 1998 (van der Giessen et al., 2001). The consequence of the invasion of raccoon dogs in the Netherlands is uncertain. The finding of *T. spiralis* in the recently arisen raccoon dog population in the Netherlands may forebode further spread of *T. spiralis* in wildlife, including rodents, increasing the risk of transmission to (outdoor kept) domestic pigs.

Conflict of interest

The authors declare that they have no competing interests.

Acknowledgements

This study was commissioned by the Netherlands Food and Consumer Product Safety Authority. The raccoon dogs have been collected in collaboration with the Dutch Wildlife Health Center and Bureau Mulder-natuurlijk. We thank all persons who contributed raccoon dogs to this study.

References

Bružinskaitė-Schmidhalter, R., Šarkūnas, M., Malakauskas, A., Mathis, A., Torgerson, P.R., Deplazes, P., 2012. Helminths of red foxes (Vulpes vulpes) and raccoon dogs (Nyctereutes procyonoides) in Lithuania. Parasitology 139, 120–127.

Eckert, J., Deplazes, P., 2004. Biological, epidemiological, and clinical aspects of echinococcosis, a zoonosis of increasing concern. Clin. Microbiol. Rev. 17, 107–135.

Eckert, J., Deplazes, P., Craig, P.S., Gemmell, M.A., Gottstein, B., Heath, D., Jenkins, D.J., Kamiya, M., Lightowlers, M., 2001. Echinococcosis in animals: clinical aspects, diagnosis and treatment. In: Eckert, J., Gemmell, M.A., Meslin, F.-X., Pawłowski, Z.S. (Eds.), WHO/OIE Manual on Echinococcosis in Humans and Animals: a Public Health Problem of Global Concern. World Organisation for Animal Health (Office International des Epizooties) and World Health Organization, Paris, pp. 73–100.

Franssen, F., Bilska-Zając, E., Deksne, G., Sprong, H., Pozio, E., Rosenthal, B., Rozycki, M., van der Giessen, J., 2015. Genetic evidence of interspecies introgression of mitochondrial genomes between Trichinella spiralis and Trichinella britovi under natural conditions. Infect. Genet. Evol. 36, 323–332.

Franssen, F., Deksne, G., Esite, Z., Havelaar, A., Swart, A., van der Giessen, J., 2014a. Trend analysis of Trichinella in a red fox population from a low endemic area using a validated artificial digestion and sequential sieving technique. Vet. Res. 45, 120.

Franssen, F., Nijsse, R., Mulder, J., Cremers, H., Dam, C., Takumi, K., van der Giessen, J., 2014b. Increase in number of helminth species from Dutch red foxes over a 35-year period. Parasit. Vectors 7, 166.

Hofer, S., Gloor, S., Muller, U., Mathis, A., Hegglin, D., Deplazes, P., 2000. High prevalence of *Echinococcus multilocularis* in urban red foxes (*Vulpes vulpes*) and voles (*Arvicola terrestris*) in the city of Zurich, Switzerland. Parasitology 120 (Pt 2), 135–142.

Isaksson, M., Hagstrom, A., Armua-Fernandez, M.T., Wahlstrom, H., Agren, E.O., Miller, A., Holmberg, A., Lukacs, M., Casulli, A., Deplazes, P., Juremalm, M., 2014. A semi-automated magnetic capture probe based DNA extraction and real-time PCR method applied in the Swedish surveillance of *Echinococcus multilocularis* in red fox (*Vulpes vulpes*) faecal samples. Parasit. Vectors 7, 583.

Kapel, C.M., Torgerson, P.R., Thompson, R.C., Deplazes, P., 2006. Reproductive potential of Echinococcus multilocularis in experimentally infected foxes, dogs, raccoon dogs and cats. Int. J. Parasitol. 36, 79–86.

Kauhala, K., Kowalczyk, R., 2011. Invasion of the raccoon dog Nyctereutes procyonoides in Europe: history of colonization, features behind its success, and threats to native fauna. Curr. Zool. 57, 584–598.

Maas, M., Dam-Deisz, W.D., van Roon, A.M., Takumi, K., van der Giessen, J.W., 2014. Significant increase of Echinococcus multilocularis prevalence in foxes, but no increased predicted risk for humans. Vet. Parasitol. 206, 167–172.

Meijer, K., Klop, E., 2014. Risicoanalyse van de Wasbeerhond in Nederland. A&W-rapport 1984. Altenburg & Wymenga ecologisch onderzoek, Feanwâlden.

Mulder, J.L., 2013. The raccoon dog (Nyctereutes procyonoides) in the Netherlands - its present status and a risk assessment. Lutra 56, 23–43.

Pannwitz, G., Mayer-Scholl, A., Balicka-Ramisz, A., Nockler, K., 2010. Increased prevalence of Trichinella spp., northeastern Germany, 2008. Emerg. Infect. Dis. 16, 936–942.

Pozio, E., Rinaldi, L., Marucci, G., Musella, V., Galati, F., Cringoli, G., Boireau, P., La Rosa, G., 2009. Hosts and habitats of Trichinella spiralis and Trichinella britovi in Europe. Int. J. Parasitol. 39, 71–79.

Schwarz, S., Sutor, A., Staubach, C., Mattis, R., Tackmann, K., Conraths, F.J., 2011. Estimated prevalence of Echinococcus multilocularis in raccoon dogs Nyctereutes procyonoides in northern Brandenburg, Germany. Curr. Zool. 57, 655–661.

Sutor, A., Schwarz, S., Conraths, F.J., 2014. The biological potential of the raccoon dog (Nyctereutes procyonoides, Gray 1834) as an invasive species in Europe—new risks for disease spread? Acta Theriol. 59, 49–59.

Takumi, K., de Vries, A., Chu, M.L., Mulder, J., Teunis, P., van der Giessen, J., 2008. Evidence for an increasing presence of Echinococcus multilocularis in foxes in The Netherlands. Int. J. Parasitol. 38, 571–578.

Takumi, K., Giessen, J.W.B.v.d, 2009. Plan Van Aanpak: Surveillance Voor Echinococcus Multilocularis in Vossen Voor 2010. National Institute for Public Health and the Environment, Bilthoven.

van der Giessen, J.W., Rombout, Y., van der Veen, A., Pozio, E., 2001. Diagnosis and epidemiology of Trichinella infections in wildlife in The Netherlands. Parasite 8, S103–S105.

van der Giessen, J.W.B., Rombout, Y., Franchimont, H.J., La Rosa, G., Pozio, E., 1998. Trichinella britovi in Foxes in the Netherlands. J. Parasitol. 84, 1065–1068.

van der Giessen, J.W., Rombout, Y.B., Franchimont, J.H., Limper, L.P., Homan, W.L., 1999. Detection of *Echinococcus multilocularis* in foxes in The Netherlands. Vet. Parasitol. 82, 49–57.