Summary:
Trichinella infections in foxes and wild boars were studied to determine the prevalence of infection in wildlife in the Netherlands. Muscles of 429 forelegs of foxes (Vulpes vulpes) and diaphragms of 11 wild boars (Sus scrofa) were artificially digested. Single larvae of Trichinella were identified at species level using random amplified polymorphic DNA (RAPD-PCR). In addition, an ELISA using ES antigen was used to test serum samples of 458 wild boars. The prevalence of Trichinella in foxes ranged from 3.9% in the eastern part of the country, 13.1% in the central part of the country and 1.3% in the western part of the country. Trichinella larvae of foxes were identified as T. britovi. In most samples, identification of larvae did not show reproducible results. The serological prevalence of Trichinella infections in wild boars was 6.8%. Wild boar populations are located in the central and in the southern part of the country. Trichinella larvae of wild boar were identified as T. spiralis. These results show that two Trichinella species are involved in the epidemiology of trichinellosis among wildlife. On the basis of previous reports, the present results suggest that the prevalence of Trichinella infection in wildlife is increasing in the last 20 years.

KEY WORDS: wildlife, T. britovi, T. spiralis, prevalence, the Netherlands.

In Europe, four Trichinella species have been described in wildlife, T. spiralis, T. britovi, T. nativa and T. pseudospiralis. Of these, T. spiralis is also able to be maintained by a domestic cycle (Pozio, 1998). The red fox, (Vulpes vulpes) is one of the main reservoirs of sylvatic trichinellosis in Western Europe and therefore it is a good indicator animal to determine the prevalence of infection in wildlife. Wild boars may be a risk to humans if trichinellosis in the sylvatic cycle occurs. In the Netherlands, the results of an active serological surveillance of pigs show that trichinellosis does not occur in the Dutch pig population since 1979 (Van Knappen, 1991). In addition, endemic trichinellosis in humans has not been observed for decades. In 1984, a survey on wildlife showed a prevalence of 4.3% in foxes and a survey between 1976 and 1980 in wild boars showed a prevalence of 1.8% (Franchimont et al., 1993). At that time larvae could not be identified at species level.

The aim of the present study was the evaluation of the prevalence of Trichinella infections in foxes and wild boar between 1996 and 2000 and the identification of the etiological agents.

MATERIAL AND METHODS
Sample collection. From 1996 until 1998, foxes from three different regions of the country were forwarded to the RIVM. Carcasses were frozen at –80°C for one week testing. After thawing, muscles of the lower parts of both forelegs were collected (Kapel et al., 1994). Two different wild boar populations were studied. One population of 750 animals originated from the central part of the country, the Veluwe. Of these animals, serum samples were collected and sent to the RIVM. The second population (about 170 animals) originated from the south east part of the country, de Roerstreek in Limburg, adjacent to Germany. Of this population, serum samples and diaphragms were sent to the RIVM.

Digestion of muscle samples
Approximately 15-30 g muscles samples of the lower forelegs of foxes and 10-30 g of the diaphragm of wild boars were digested using Trichromatic 35°. Single muscle larvae of Trichinella were kept in 5 µl of water at –20°C.

Identification of muscle larvae
Individual muscle larvae were identified by RAPD-PCR (Bandi et al., 1995).

SEROLOGY
Serum samples of wild boar were tested by means of an ELISA using Excretory/Secretory antigen (van Knappen et al., 1986).
Trichinella in foxes

Fig. 1. - Spatial distribution of *Trichinella* in foxes in the Netherlands. Negative (○) and positive (△) *Trichinella* foxes as found in various regions in the Netherlands. The human population densities are marked on the map.

Source: RIVM, CBS
RESULTS

A total of 429 foxes was examined. The number of animals investigated from the different areas and the number of positive animals are listed in Table 1. In the border area with Germany and Belgium, 11 foxes (3.9 %) were found positive for *Trichinella* larvae, with a worm burden ranging from 0.04 to 0.71 larvae per g (LPG) (Van der Giessen et al., 1998). In the Veluwe region, 10 foxes (13.1 %) were positive for *Trichinella* larvae with a worm burden ranging from 0.04 to 0.6 LPG. In the coastal region, only one animal was positive (1.3 %; 0.2 LPG). The spatial distribution of all foxes examined and the location of the *Trichinella*-positive foxes is shown in Figure 1. *Trichinella* positive foxes were even found in areas with a high human population density. Larvae from foxes were identified as *T. britovi* (Van der Giessen et al., 1998). A seroprevalence of 6.8 % (31/458) was detected in wild boars. Two diaphragms of 11 wild boars from the Roerstreek region were positive (0.2 and 0.3 LPG) by artificial digestion. Larvae were identified as *T. spiralis*.

DISCUSSION

Comparing the results of this study to those from the study in foxes in the Netherlands in 1984, the overall prevalence is similar to the 4.3 % found in 1984 (Franchimont et al., 1993). However, at that time foxes were collected only in the central part of the country, the Veluwe. The estimated prevalence of *Trichinella* infections of foxes derived from the Veluwe in 1998 is 13.1 %. This suggests a significant increase of the prevalence since 1984. One but probably not the whole explanation for this increase could be the different target sample. In fact, we examined forelegs, whereas muscle samples of the tongue were examined in 1984. The occurrence of *T. britovi* in the Netherlands, one of the most densely populated countries in Europe, suggests a different epidemiological scenario compared to the one that was previously described in other European countries, where *T. britovi* was found only in remote wilderness and in mountain areas (Pozio, 1998). Between 1976 and 1980, a seroprevalence of 1.8 % was detected in wild boars from the Netherlands (Franchimont *et al.*, 1993), whereas the seroprevalence increased to 6.8 % in the present study, suggesting a greater diffusion of this parasite among wild boars. The presence of *T. britovi* in the fox population of the Netherlands appears to be without consequences for modern commercial pig farming (Van Knapen, 1991). However, the presence of *T. spiralis* in wild boars and the higher prevalence of *Trichinella* infection than that detected in the past, suggests that an increasing risk exists in the Netherlands of the establishment of new foci of domestic trichinellosis mainly in organic pig farms. In conclusion, sylvatic trichinellosis occurs in the Netherlands even in areas with a high human population density. These results add new information on the epidemiology of sylvatic trichinellosis and should be considered when implementing new strategies to control this zoonosis in Europe.

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