

## DIAGNOSIS AND EPIDEMIOLOGY OF *TRICHINELLA* INFECTIONS IN WILDLIFE IN THE NETHERLANDS

VAN DER GIESSEN J.W.B.\*, ROMBOUT Y.\*, VAN DER VEEN A.\*\* & POZIO E.\*\*\*

### 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 the eastern part of the country, 13.1 % in the central part of the country and 1.3 % in the most 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 Knapen, 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  $-80^{\circ}\text{C}$  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 Trichomatic 35<sup>®</sup>. Single muscle larvae of *Trichinella* were kept in 5  $\mu\text{l}$  of water at  $-20^{\circ}\text{C}$ .

### 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 Knapen *et al.*, 1986).

\* Microbiological Laboratory for Health Protection.

\*\* Department for Public Health Forecasting, National Institute of Public Health and the Environment (RIVM), Antonie van Leeuwenhoeklaan 9, 3720 BA Bilthoven, The Netherlands.

\*\*\* Department of Parasitology, Istituto Superiore di Sanità, Viale Regina Elena 299, 00161 Rome, Italy.

Correspondence: J.W.B. van der Giessen.  
Tel.: +3130-2743926 – Fax: +3130-2744434.  
e-mail: joke.van.der.giessen@rivm.nl

## Trichinella in foxes

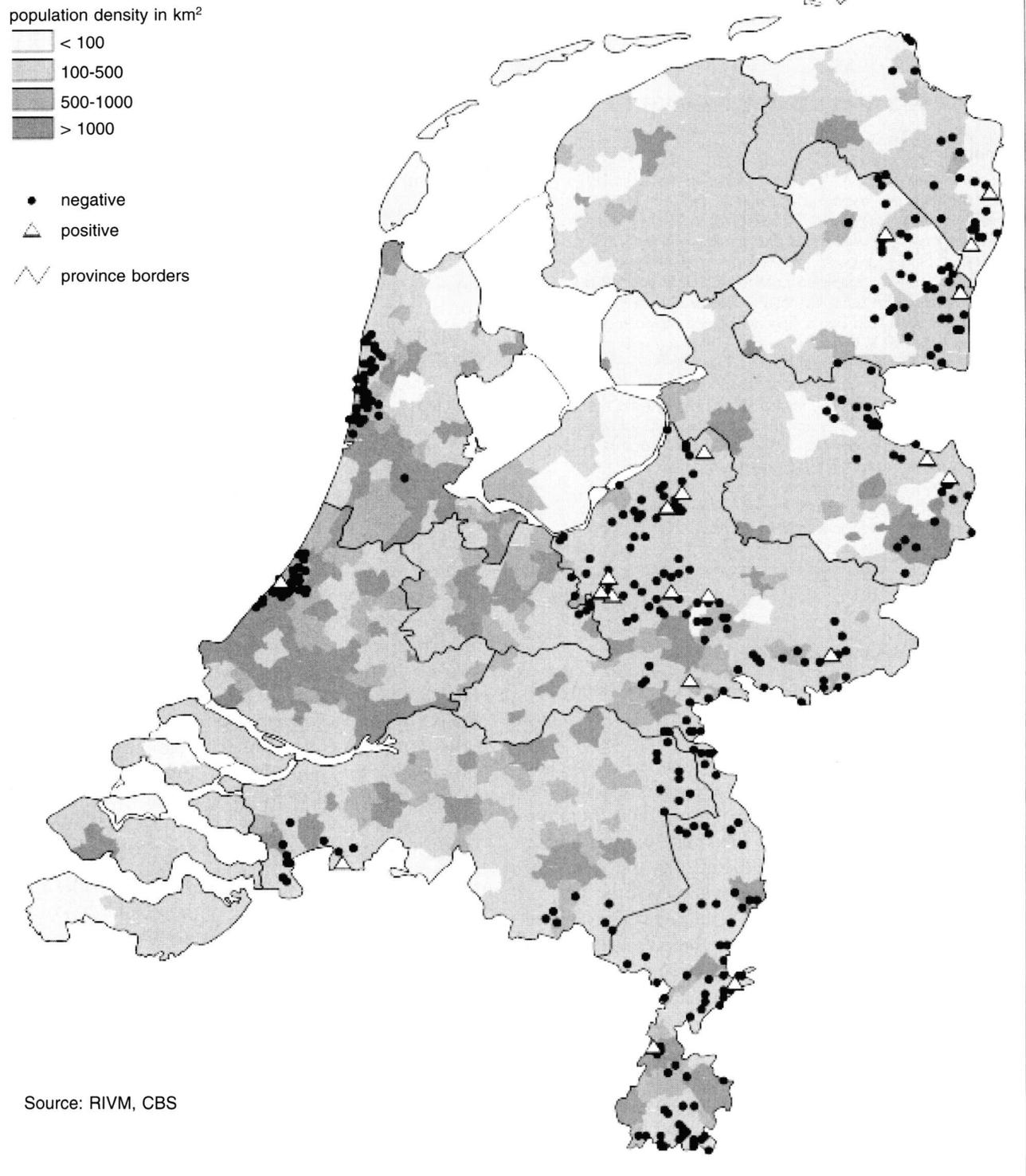


Fig. 1. – Spatial distribution of *Trichinella* in foxes in the Netherlands. Negative (●) and positive (Δ) *Trichinella* foxes as found in various regions in the Netherlands. The human population densities are marked on the map.

Region investigated	Number of positive foxes by total number tested (%)
Border region	11/276 (3.9)
Veluwe (central part)	10/76 (13.1)
Coastal region	1/77 (1.3)
Total number of foxes	22/429 (5.1)

Table 1. – Prevalence of trichinellosis in vulpine populations from three regions of the Netherlands.

## 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.

## ACKNOWLEDGEMENTS

We thank Margriet Montizaan (Royal Dutch Shooting Society), Bernard Arends (Veterinary Public Health Inspection) and participating hunters for the collection of the foxes. Frans van Knapen for critical reading of the manuscript and the helpful discussions. This study was carried out on behalf of the Veterinary Public Health Inspection of the Ministry of Public Health.

## REFERENCES

- BANDI C., LA ROSA G., BARDIN M.G., DAMIANI G., COMINCINI S., TASCIOTTI L. & POZIO E. Random amplified polymorphic DNA fingerprints of the eight taxa of *Trichinella* and their comparison with allozyme analysis. *Parasitology*, 1995, 110, 401-407.
- FRANCHIMONT J.H., VAN KNAPEN F. & KREMERS A.F.T. Trichinellosis in wildlife in the Netherlands. Proceedings ICT 8, 1993, 561-564.
- KAPEL C.M., HENRIKSEN S.A., DIETZ H.H., HENDRIKSEN P. & NANSEN P. A study on the predilection sites of *Trichinella spiralis* muscle larvae in experimentally infected foxes (*Alopex lagopus*, *Vulpes vulpes*). *Acta Veterinaria Scandinavica*, 1994, 35, 125-132.
- POZIO E. Trichinellosis in the European Union: Epidemiology, ecology and economic impact. *Parasitology Today*, 1998, 14, 35-38.
- VAN DER GIESSEN J.W.B., ROMBOUT Y., FRANCHIMONT H.J., LA ROSA G. & POZIO E. *Trichinella britovi* in the Netherlands. *J. Parasitol.*, 1998, 84 (5), 1065-1068.
- VAN KNAPEN F., BUIJS J. & RUITENBERG E.J. *Trichinella spiralis* antibodies. In: Bergmeyer H.U., Bergmeyer J. & Grassl M. (eds). Methods of enzymatic Analysis. 3<sup>rd</sup> edition. VCH, Weinheim, FRG, 1986, 393-406.
- VAN KNAPEN F. Absence of trichinellosis in the Netherlands. RIVM Report 1991 nr. 188802002.