Veterinary Parasitology 163 (2009) 93–100

Contents lists available at ScienceDirect

Veterinary Parasitology

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

Serological detection of anti-*Trichinella* antibodies in wild foxes and experimentally infected farmed foxes in Norway

Rebecca K. Davidson [a,*], Irene Ørpetveit [b], Lone Møller [c], Christian M.O. Kapel [c]

[a] Section for Wildlife Diseases, National Veterinary Institute, P.O. Box 750 Sentrum, NO-0106 Oslo, Norway
[b] Section for Virology and Serology, National Veterinary Institute, P.O. Box 750 Sentrum, NO-0106 Oslo, Norway
[c] Danish Centre for Experimental Parasitology, Section for Zoology, Department of Agronomy and Ecology, Faculty of Life Sciences, University of Copenhagen, DK-1871 Frederiksberg C, Denmark

ARTICLE INFO

Article history:
Received 31 October 2008
Received in revised form 4 March 2009
Accepted 9 March 2009

Keywords:
Antibody persistence
Excretory/secretory antigen
Indirect ELISA
Seroprevalence
Surveillance
Trichinellosis
Trichinella nativa
Tyvelose antigen
Western blot
Wildlife

ABSTRACT

Trichinella surveillance in wildlife has relied on the detection of muscle larvae using digestion techniques. Serology has been proposed as more suitable for large-scale epidemiological studies in wildlife. In this study, 328 individual sera from wild red foxes and 16 sera from experimentally infected farmed foxes were serologically tested with both excretory/secretory antigen (E/S) and the synthetic β-tyvelose glycan antigen, in indirect ELISA tests. The wild red foxes (*Vulpes vulpes*) had previously been examined for muscle larvae, using muscle digestion, whilst the experimentally infected farmed foxes were inoculated per os with either a low dose, 500 larvae, or a high dose, 10,000 of *Trichinella nativa* muscle larvae. Western blot (WB) was carried out on all seropositive samples using crude larval antigen.

The present study found both β-tyvelose and E/S antigen suited for the detection of antibodies to *Trichinella* spp., and *T. nativa* in particular, in foxes. Both ELISA antigens performed well, although, the E/S antigen was superior to the β-tyvelose antigen, with sera that had been stored at −20 °C for more than 10 years. Neither antigen, however, detected all of the samples proven seropositive by WB: E/S detected 21 of the 27 wild red fox sera positive by WB; β-tyvelose detected 22 positive sera; and in total 24 of the 27 positive WB sera were identified using both antigens. Serology alone, without WB or muscle digestion, led to a two- to threefold higher seroprevalence estimate, respectively. The use of E/S antigen in conjunction with the WB was the method of choice for the screening of wild red fox populations for *Trichinella*. Antibody persistence to *T. nativa* was short in the low dose group where antibody levels were not different from background by 32 wpi. In total, 7.3% (24/328) of the wild red fox population had antibodies to *Trichinella* on ELISA and WB. Antibodies were identified in foxes from a further two regions in Norway compared to the original muscle digestion results.

1. Introduction

Surveillance for *Trichinella* in wildlife population is regarded as a tool for risk assessment in domestic animal production (European Community, 2005) and serological tests have been proposed as suitable for such purposes (Nöckler and Kapel, 2007). Host antibodies produced against one species of *Trichinella* are able to detect antigens from other *Trichinella* species (Gamble et al., 2004; Kapel and Gamble, 2000; Nöckler et al., 2005) and as such species-specific *Trichinella* antigens are not required for serological assays. Excretory/secretory (E/S) antigen and

* Corresponding author. Tel.: +47 23216352.
E-mail address: rebecca.davidson@vetinst.no (R.K. Davidson).

0304-4017/$ – see front matter © 2009 Elsevier B.V. All rights reserved.
doi:10.1016/j.vetpar.2009.03.020

the synthetic β-tyvelose glycan antigen have been used when carrying out *Trichinella* ELISA analysis. Both crude larval antigen and E/S antigen have been used in WB assays (Ortega-Pierres et al., 1996) however the somatic components of crude larval antigen cross-react with other helminths rendering it unsuitable for ELISA (Bruschi, 2002; Gamble et al., 2004).

E/S antigen has been used to investigate seroprevalence in wild red foxes and found prevalence levels 10 times higher than that seen with muscle digestion in the same population (Gottstein et al., 1997; Vercammen et al., 2002; Wacker et al., 1999). These studies posited that this higher seroprevalence was either related to the increased sensitivity of the method or to reduced specificity. None of the currently published studies in wild red foxes has carried out Western blot (WB) nor has the β-tyvelose antigen been tested in this species. It has been evaluated in other species and in experimentally infected foxes (Møller et al., 2005a; Nöckler et al., 1995; Pozio et al., 2002).

The aim of this study was to investigate if the β-tyvelose and E/S antigens would yield comparable results in wild red foxes and if serology could be a suitable alternative to muscle digestion for *Trichinella* surveillance in this species. Antibody dynamics vary depending on host species, *Trichinella* genotype and infection dose (Kapel and Gamble, 2000; Malakauskas et al., 2001; Nöckler et al., 2000). The persistence of *T. nativa* antibodies, the most commonly found genotype in Norway (Davidson et al., 2006), was investigated in experimentally infected farmed foxes using E/S and synthetic β-tyvelose antigens. Serum from Norwegian wild red foxes, which had previously been investigated for *Trichinella* muscle larvae using digestion methods (Davidson et al., 2006), were then investigated using both antigens in an indirect ELISA and crude larval antigen in a WB.

2. Materials and methods

2.1. Foxes and Trichinella infections

Serum for the present study originated from three groups of foxes (I–III):

(I) Farmed blue foxes (*Vulpes lagopus* previously *Alopex lagopus*) ($N = 8$) inoculated per os with minced muscle containing approximately 500 *T. nativa* larvae (low dose) originating from four of the naturally infected Norwegian red foxes (*Vulpes vulpes*) described in group III, and subsequently maintained in farmed blue foxes. One of the isolates had been passaged in six farmed foxes since it was first isolated in 1994 whilst another had undergone two passages and the remaining two isolates had been passaged just once in farmed foxes prior to this study. Blood was sampled both prior to inoculation with the larvae and at 5, 10, 20, 32 and 43 weeks post-inoculation (wpi). Two farmed blue foxes were sacrificed at 32 wpi to provide larvae with which to inoculate the second group of foxes (II) (Davidson et al., 2008) whilst the remaining animals were euthanised at 43 wpi.

(II) Larvae of three *T. nativa* isolates; two from foxes in Norway and one originating in Alaska and maintained in mice (1, 2 or 3: ISS1607 (Norway, *Vulpes vulpes*); ISS1606 (Norway, *Vulpes vulpes*); and ISS042 (Alaska, USA, *Ursus maritimus*)) were released by HCl–pepsin digestion and 10,000 larvae (HD) of isolate 1, 2 or 3 were administered by stomach gavage to farmed silver foxes (*Vulpes vulpes*) ($N = 8$). Blood samples were obtained prior to inoculation, and at 5, 10 and 20 wpi, after which all the foxes were euthanised.

(III) The wild red fox population examined is the same as that described by Davidson et al. (2006). Briefly, hunters supplied fox carcasses during two separate study periods, 1994–1995 ($N = 65$) and 2002–2005 ($N = 328$). In total, 393 red fox carcasses were submitted, however, serum samples were insufficient in 65 animals, 9 from the first study period and 56 from the second, leaving a total of 328 red foxes in the study group. The sex, age and geographic origin of each animal were recorded at post-mortem.

The cephalic vein was used for *in vivo* sampling of the experimentally infected foxes, whilst blood was obtained, at necropsy, from the base of the heart, femoral artery or caudal vena cava from the wild red foxes. All the blood samples were centrifuged for 3 min at 3000 rpm and the serum stored at −20 °C until further examination. Muscle larvae burden was assessed at necropsy using HCl–pepsin digestion of a single sample containing a minimum of 10 g of quadriceps muscle (Kapel et al., 2005). Muscle larvae in the wild red foxes had previously been identified to species level and *Trichinella britovi* and *T. nativa* identified (Davidson et al., 2006). The farmed foxes were fed with standard rations throughout the study with the temporary exception of the low dose (LD) group which were starved overnight prior to inoculation and received no additional food until all the minced muscle had been consumed. The foxes were euthanised by administrating a short electrical shock. The foxes in both groups were treated within ethical guidelines for experimental animals and approval for both the LD and HD infection groups was given by the Norwegian Committee for Experimental Animals (licence number S228/02).

2.2. Antibody detection

Two different antigens were used: the E/S antigen, produced according to Kapel and Gamble (2000), diluted 1:4000, or the synthetic glycan antigen β-tyvelose (Heska Corporation, Fort Collins, Colorado) diluted 1:800. Detection of specific antibody from the serum was detected with an alkaline phosphatase conjugated goat anti-canine IgG antibody (Sigma–Aldrich), diluted 1:1000. The ELISA was carried out as described by Møller et al. (2005a). All samples, where possible, were run once using the E/S antigen and once with the β-tyvelose antigen. Twelve serum samples were examined using the E/S antigen only, due to an insufficient volume of serum.