Comparison of two antigens for demonstration of *Trichinella* spp. antibodies in blood and muscle fluid of foxes, pigs and wild boars

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Abstract

For the surveillance of trichinellosis, the digestion method is reliable but also labour intensive. The serological methods for the detection of *Trichinella*-specific antibodies using ELISA offer a sensitive and relatively specific alternative. For serological studies, sera or plasma from blood samples are the most common source of antibodies, but although the concentration of antibodies is approximately 10-fold lower, muscle fluid can be a good alternative particularly for testing of wildlife samples. In the present study, an indirect ELISA technique was evaluated on both sera and muscle fluids from experimentally infected foxes, pigs, and wild boars using both excretory/secretory (E/S) antigens and a synthetic glycan antigen, β-tyvelose. Although the synthetic antigen appears to be less sensitive than the E/S antigens, *Trichinella*-specific IgG antibodies were detected in both serum samples and muscle fluid samples from pigs, wild boars and foxes infected at levels which would be important for food safety or represent a significant reservoir for further transmission.

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1. Introduction

Although several methods for the detection of *Trichinella* in meat are available (Gamble, 1998; Nöckler et al., 2000) the magnetic stirrer digestion method is considered the gold standard (Gamble et al., 2000). Enzyme linked immunosorbent assay (ELISA) for the detection of circulating antibodies against *Trichinella* in blood samples has proven to be a highly sensitive test for the detection of *Trichinella* spp. in pigs and wild boars (van Knapen et al., 1981; Flores-Trujillo et al., 1995; Gamble et al., 1996; Gamble, 1998; Kapel and Gamble, 2000; Kapel, 2001) having the advantage of being rapid and relatively inexpensive. For the analysis of *Trichinella*-specific anti-
bodies, serum or plasma is usually preferred, but muscle fluid may offer a good alternative for testing game meat products, although antibodies are present at lower levels compared to serum. Thus, even if serological testing is still not suitable for meat inspection, it is a powerful tool for wildlife surveillance. The aim of the present study was to evaluate an indirect ELISA technique on both sera and muscle fluids from experimentally infected pigs, foxes and wild boars, employing two highly specific Trichinella antigens.

2. Materials and methods

2.1. Experimental design, animals and inoculations

Blood serum and muscle fluids were collected from 102 pigs (Kapel and Gamble, 2000), 36 wild boars (Kapel, 2001) and 108 foxes (data not published). The pigs were divided into eight groups of 14 animals except for the group infected with T. pseudospiralis (ISS 141) which contained only four animals. The foxes and wild boars were divided into nine groups. The foxes contained with 12 animals in each group and wild boars had only four animals per group. The animals were experimentally infected in groups with one of the following Trichinella genotypes, all registered at the International Trichinella Reference Centre in Rome; T. spiralis (ISS 004), T. nativa (ISS 042), T. britovi (ISS 100), T. pseudospiralis (ISS 013, ISS 470, ISS 141), T. murrelli (ISS 035), Trichinella (T6) (ISS 034), and T. nelsoni (ISS 037). All animals were inoculated with 10,000 larvae at 10 weeks of age (pigs and foxes) and 25–30 weeks of age (wild boars). No pigs were inoculated with T. pseudospiralis (ISS 470).

2.2. Serum and muscle fluids collection

Blood was collected prior to inoculation, then weekly or twice monthly up to 40 weeks post inoculation (wpi) for pigs and foxes, and weekly up to 11 wpi for wild boars and sera was isolated by centrifugation after clotting. At slaughter, muscle samples from all animals were taken for fluid extraction and for digestion to detect Trichinella larvae (data not shown). Muscle fluid extraction was done according to Kapel et al. (1998), and both serum and muscle fluid samples were kept in Eppendorf tubes at −20 °C until tested for antibodies.

2.3. ELISA and antigens

Two different antigens, an excretory/secretory (E/S) antigen and a 3,6-dideoxyhexose glycan (β-tyvelose antigen) coupled with BSA (Bruschi et al., 2001), was used in an indirect ELISA to detect Trichinella-specific IgG antibodies. The β-tyvelose antigen was purchased from Heska Corporation (Fort Collins, Colorado) while the E/S antigens were isolated from different Trichinella species according to Kapel and Gamble (2000). Both antigens were adjusted to 1 mg/mL and stored at −20 °C until used.

Briefly, the E/S antigens (0.25 μg/mL) and the β-tyvelose antigen (1.25 μg/mL) were prepared in carbonate buffer, and 100 μL/well were added to microtitre plates and left overnight at 4 °C. Plates were washed and incubated with either serum samples diluted 1:1000 (pigs) or 1:200 (wild boars and foxes), or muscle fluid samples diluted 1:200 (pigs, foxes and wild boars) for 1 h at room temperature. For pig and wild boar samples, an anti-pig IgG alkaline phosphatase labelled conjugate (KPL) was used as second antibody. For fox samples, an anti-dog IgG alkaline phosphatase labelled conjugate (Sigma) was used. Both conjugates were diluted 1:1000 and 100 μL were added to each well and the plates were incubated for 1 h at room temperature. A substrate (BIO-RAD) was used and after 30 min the reaction was stopped by adding 100 μL/well 1 M NaOH. Results were read at 405 nm using an ELISA-reader (Multiscan EX, Labsystems). The positive cut-off values for the serum samples were calculated according to the following formula: (positive control/negative control) × 0.15 + negative control (Kapel and Gamble, 2000). No control samples of muscle fluid were available for calculation of cut-off values.

3. Results

Trichinella-specific IgG antibodies were detected in both serum and muscle fluid of all experimental groups (Fig. 1a–f; data only shown for T. spiralis, T. nativa and T. britovi) lower antibody levels were
generally detected in muscle fluids. No positive reactions were seen in serum samples taken before inoculation, and for all host species, sero-conversion was observed approximately 3 wpi for both antigens. Antibody detection appeared more consistent using the E/S antigens in all three animal groups, while they were more variable using the β-tyvelose antigen (Fig. 1a–c versus d–f). Some differences were observed in the absorbance levels in the muscle fluid samples, but the readings were comparable using the two different antigens in all three host species. The sensitivity of the antigens in detecting infection with *Trichinella* spp. in the serum samples was 99–100% for the E/S antigens and 90–95% for the β-tyvelose antigen. Thus of 246 animals, 17 were not detected by the β-tyvelose antigen at any time during the infection. Ten were pigs with low muscle larval burdens (0.011–19.2 lpg). In comparison, only one animal (pig) with a low infection (0.08 lpg) was not detected using the E/S antigens.

4. Discussion

The present experimental-infections of pigs, wild boars and foxes revealed that *Trichinella*-specific antibodies can be detected in both serum and muscle fluid by both E/S and β-tyvelose antigens, although at a lower level in the muscle fluids. This dilution effect has been demonstrated previously by Kapel et al. (1998) who found approximately 10 times lower
concentrations of antibody in muscle fluids than in sera. Unfortunately, no control muscle fluid samples were available for calculation of cut-off values which prevents an evaluation of the sensitivity of the muscle fluid assay.

Although muscle fluids appear to offer a suitable matrix for sero-surveillance in wildlife, its use for monitoring pig herds requires an optimization of the muscle fluid dilutions and a determination of a positive cut-off value. As shown previously (Kapel and Gamble, 2000; Kapel, 2001), low infection levels in pigs and wild boars (e.g. T. nativa infections with less than 0.001 lpg) are still capable of inducing sero-conversion, although antibody levels show a marked decrease over periods of weeks. In part, this may explain the variability observed in antibody responses to β-tyvelose antigen and to a lesser extent to E/S antigens observed for the different Trichinella genotypes.

The indirect ELISA method is commonly used for surveillance of Trichinella; however, its specificity and sensitivity relies largely on antigen chosen for the assay (Mahannop et al., 1995; Pozio et al., 2002; Gamble et al., 2004). In pigs, E/S antigens have previously been demonstrated to have a higher specificity than somatic antigens (Zarlenga and Gamble, 1990), and the β-tyvelose antigen has offered a synthetic alternative to E/S antigens in ELISA for diagnosis of trichinellosis in pigs (Gamble et al., 1997). In contrast, the used of both E/S antigens and β-tyvelose antigen to evaluate infections in horses resulted in false-positive results (Pozio et al., 2002). Although the present study is limited in many ways, the lower sensitivity observed when using the β-tyvelose antigen relative to the E/S antigens indicates that the β-tyvelose antigen may not be suitable for evaluation of pig herds; however, it may be adequate for surveillance in certain wildlife species where specificity of detection is more relevant. For surveillance among domestic pigs, the use of E/S antigens combined with a confirmatory Western blot assay appears more appropriate.

References


