The Veterinary Journal 196 (2013) 439–444

Contents lists available at SciVerse ScienceDirect

The Veterinary Journal

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

A bead-based suspension array for the serological detection of Trichinella in pigs

F.J. van der Wal *, R.P. Achterberg, A. Kant, C.B.M. Maassen [1]

Central Veterinary Institute, Wageningen UR, Lelystad, The Netherlands

ARTICLE INFO

Article history:
Accepted 19 October 2012

Keywords:
Trichinella spiralis
Serology
Pig
Suspension array
Human
Zoonosis

ABSTRACT

The feasibility of using bead-based suspension arrays to detect serological evidence of Trichinella in pigs was assessed. *Trichinella spiralis* excretory–secretory antigen was covalently coupled to paramagnetic beads and used to bind serum antibodies, which were subsequently detected using anti-swine antibody. The assay was evaluated by testing pig sera from farms where trichinellosis was endemic and comparing the results with those obtained using two commercially available ELISAs. With cut-offs established by receiver operating characteristic (ROC) analysis, digestion-negative sera from a Trichinella-free population of pigs were deemed seronegative. When anti-swine antibody was replaced with protein A/G, higher test sensitivity (94% vs. 88%) at similar test specificity (95%), was achieved. The potential use of this assay in species other than swine was also demonstrated by testing human sera.

Introduction

Trichinellosis is a parasitic zoonosis reported to affect as many as 11 million people worldwide (Dupouy-Camet, 2000). The disease is caused by nematodes of the genus *Trichinella* of which *Trichinella spiralis* is the species most commonly implicated in human infections (Pozio and Murrell, 2006). Infection occurs through the ingestion of raw or undercooked meat, and the clinical sequelae for humans can be severe (Capo and Despommier, 1996). While *Trichinella* spp. can be found in many different hosts (Pozio, 2005), the major reservoirs for human infection are domestic pigs, wild boar, and horses (Pozio and Murrell, 2006). While in North America and most of Western Europe infections due to *Trichinella* spp. are rare in domestic animals, in other parts of the world infection of the local animal populations is endemic (Gottstein et al., 2009).

In order to control trichinellosis, the meat from >167 million pigs is subjected to mandatory inspection within the EU annually (Alban et al., 2011). This involves the artificial digestion and microscopic examination of pooled meat samples from 100 pigs to look for larvae. When such a 'pool' is deemed positive, individual samples are investigated by artificial digestion (Nöckler et al., 2000). Serological screening, using the excretory/secretory (E/S) antigen of *T. spiralis* larvae in an ELISA (Gamble et al., 1988; Nöckler et al., 1995), has been used and can be more sensitive than artificial digestion (Gajadhar et al., 2009). However, as there is an inevitable lag between serological positivity and infection, the larval burden may not always be reflected in the test result (Nöckler et al., 1995). Nevertheless, serology can be used to monitor trichinellosis-free herds or seronegative populations in low-risk regions (Commission Regulation EC 2075/2005). In this context the use of serology to contemporaneously screen for this and other zoonotic pathogens such as *Salmonella* spp. (Wegener et al., 2003) in a multiplex format is an appealing goal.

Although there are many examples illustrating bead-based assays can be used for multiplex serology in humans (Dias et al., 2005; van Gageldonk et al., 2008; Casabonne et al., 2009; Antonsson et al., 2010), the use of bead-based serology for veterinary applications is limited (Clavijo et al., 2006; Perkins et al., 2006; Go et al., 2008; Watson et al., 2009; Anderson et al., 2011; van der Wal et al., 2012). In this study, we first investigated whether a serological test for Trichinella infection in pigs could be established in a format suitable for multiplexing. The Luminex platform was selected as it allows the use of multiple antigens in bead-based suspension arrays (Perkins et al., 2006; Krishhan et al., 2009). Our second objective was to investigate if the use of bead-based serology was possible with an immunoglobulin binding protein such as protein A/G (Inoshima et al., 1999; Zhang et al., 2010) in order to create an assay that could potentially be used in multiple species, as previously described using protein A (Gamble et al., 1983).

We compared the results of our candidate assay with those of an ELISA using sera from experimentally infected pigs, as well as from pigs from Trichinella-endemic and -free populations. In addition, the use of protein A/G instead of an anti-swine antibody was evaluated with both porcine and human sera to investigate if the assay could be used in more than one species.

* Corresponding author. Tel.: +31 320 238395.
E-mail address: fimme.vanderwal@wur.nl (F.J. van der Wal).
[1] Present address: National Institute for Public Health (RIVM), Centre for Infectious Disease Control, Laboratory for Zoonoses and Environmental Microbiology, Bilthoven, The Netherlands.

1090-0233/$ - see front matter © 2012 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.tvjl.2012.10.029

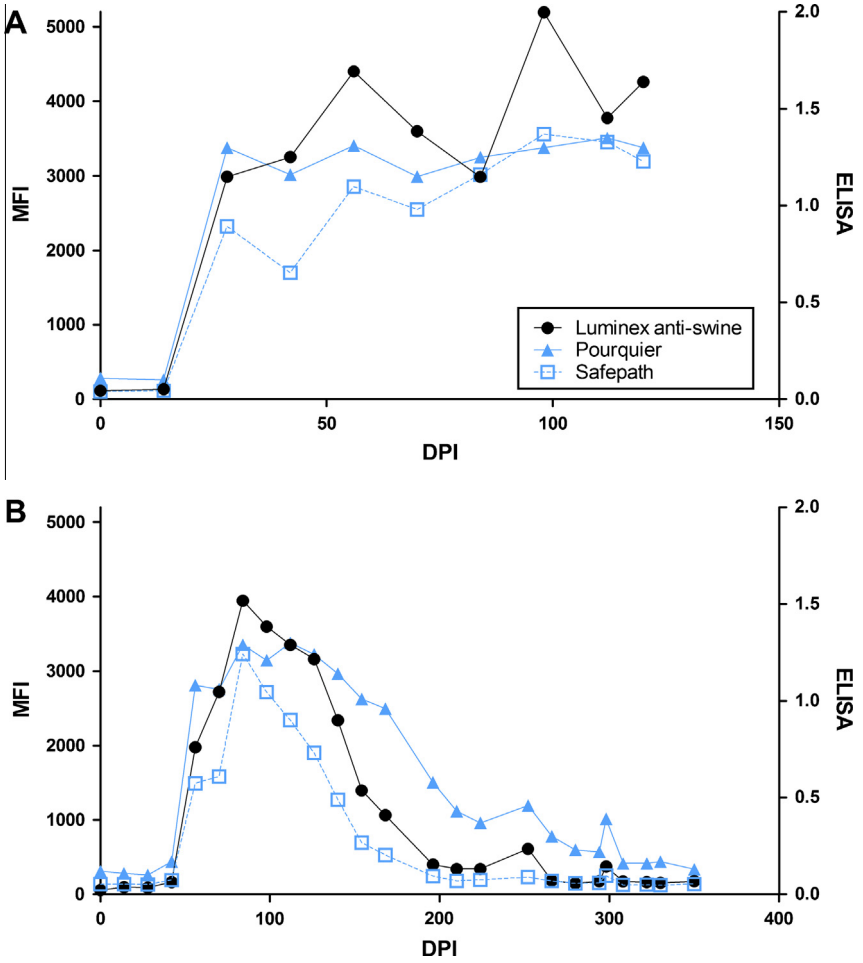

Fig. 1. Graphs A and B illustrating the performance of bead-based Trichinella assay (BBA, Luminex anti-swine) in pigs relative to two commercially available ELISAs (Pourquier and Safepath) using longitudinal serum samples from two pigs (graphs A and B), experimentally infected with 250 *T. spiralis* larvae. Signal strength of the bead-based assay (expressed as median fluorescence intensity [MFI], left *Y*-axis) is plotted against days post-infection (DPI). The results of the two ELISAs are detailed on the right *Y*-axis: ranging from 0% to 200% for the Pourquier, and at a range of optical densities between 0 and 2 for the Safepath ELISA, respectively. Both sets of ELISA results are presented ranging from 0 to 2.

Materials and methods

Selection of serum samples

Sera of pigs infected with 100, 200, 1000 and 10,000 *T. spiralis* larvae (days 83, 84, 92, and 92 post-infection [p.i.], respectively) were kindly donated by Dr. M. Swanenburg and used to establish the assay. A longitudinal series of sera from two pigs infected with 250 larvae were kindly provided by Dr. R. Gamble. The average number of larvae/g (LPG) in samples of diaphragm at necropsy were 2.67 (day 120 p.i.) and 0.07 (day 365 p.i.), respectively (R. Gamble, personal communication). Sera from pigs experimentally infected with different amounts of *T. spiralis* larvae were obtained from Dr. K. Nöckler (Nöckler et al., 1995). For examination of cross reactivity, sera from pigs infected with *Trichuris suis* ($n = 6$), *Toxoplasma gondii* ($n = 5$), and *Ascaris suum* ($n = 12$), were kindly provided by Drs. H. Kringel, J. Cornelissen, F. Borgsteede, and sera from pigs infected with *Sarcoptes scabiei* ($n = 3$), *Dermatophagoides pteronysinnus* ($n = 2$), and *Acarus siro* ($n = 2$), were kindly provided by Dr. H. van der Heijden.

The work was approved by the institute's Animal Experiments Committee (DEC) in accordance with Dutch regulations (DEC reference numbers: *T. spiralis*, 2006148.a and 2007079.a; *Toxoplasma gondii*, 2006105.b; *Ascaris suum*, 2006134.a and 2007154.a).

To evaluate the assay, a set of 244 swine sera collected in endemic regions of Argentina (Döpfer et al., 2006; Teunis et al., 2009), and a set of 120 sera from digestion-negative pigs (seronegative by ELISA [Maassen et al., 2007]), were used. To test the bead-based assay using protein A/G in species other than swine, 30 Trichinella seronegative and 31 Trichinella seropositive human sera were kindly provided by Drs. T. Garate and E. Rodriguez. The serological status of these samples had been established using indirect immunofluorescence (Sulzer, 1965) and ELISA (Escalante et al., 2004). The human samples had already been collected as part of public health diagnostic activities and had been submitted to both 15/1999 (Spanish Law) and 1720/2007 (Spanish Royal Order on Data Protection). Control (negative) samples were obtained from volunteers after they had provided written informed consent.

Coupling of E/S antigens to carboxylated beads

Excretory–secretory antigen, produced as previously described (Franssen et al., 2011), was obtained from Dr. J. van der Giessen; 13.75 μg was covalently coupled to 2.5×10^6 carboxylated paramagnetic beads (MagPlex microspheres, Luminex). Coupling was achieved through a generic two-step carbodiimide coupling with sulfo-*N*-hydroxysulfosuccinimide (NHS) and 1-ethyl-3-[3-dimethylaminopropyl]carbodiimide hydrochloride (EDC) (Pierce) according to the manufacturer's instructions.

Bead-based serology

Assays were performed with a Luminex 200 system (Luminex) using 1000 beads/50 μL PBS-T (0.05% Tween 20). Aliquots of 50 μL bead mix and 50 μL diluted serum (1:200 in PBS-T as established in pilot experiments) were mixed and incubated in a 96-well plate for 30 min in the dark at room temperature on a plate shaker (at approximately 600 rpm). After incubation, the plate was placed on a magnet (Dynal MPC-96-S; Invitrogen) for 1 min after which the beads were washed with 100 μL of PBS-T. Next, 100 μL of biotinylated secondary antibody (goat anti-swine IgG, catalogue number. 114-065-003, Jackson ImmunoResearch) in PBS-T were added (1:5000). Alternatively, biotinylated protein A/G (1:5000 of 430 μg/mL) was used: 500 μg of recombinant protein A/G (Cat. No. 21186, Pierce) was biotinylated using EZ-Link Sulfo-NHS-Biotin (Pierce) in a 1:1 ratio, according to the manufacturer's instructions.