Comparison of two commercial ELISA kits and magnetic stirrer method for detection of Trichinella spp. in a pig slaughterhouse

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Abstract

ELISA represents a useful rapid method to detect the presence of specific antibodies on serum, plasma or meat juice collected at slaughter, however, false- and positive-results may occur depending on the sensitivity and specificity of the test. In this study we compare two commercial ELISA kits for the detection of specific antibodies against Trichinella spp. with respect to the gold standard method (artificial digestion) in a pig slaughterhouse. A total of 709 Iberian pigs belonging to 79 free-range herds were randomly selected and sampled (five to ten animals/herd) (Win Episcope 2.0; 95% confidence level, 8% accepted error). Blood samples were collected at the slaughterhouse, and serum was harvested and frozen at -80 ºC until testing. Sera samples were analyzed for the detection of specific anti-Trichinella spp. antibodies by means of two commercial ELISA kits, following manufacturer's instructions (PrioCHECK® Trichinella Ab, Prionics; ID Screen® Trichinella Indirect, IDVet Innovative Diagnostics; cut-off > 15% and > 50%, respectively). Samples from the diaphragm pillar (1 gram/animal) were collected and subjected to artificial digestion method for pooled sample digestion (100 g/pool) following the regulation EC-2075/2005. Specific anti-Trichinella spp. antibodies with PrioCHECK® ELISA were detected in 3 out of 709 animals, belonging to 3 out of 79 herds. Nonetheless, all the sampled animals displayed negative results for both IDScreen® ELISA and artificial digestion. The positive results observed with the first ELISA may be related to a higher sensitivity, being able to detect contact but not infestation with the parasite. Although both ELISA kits are coated with Trichinella E/S antigen, differences in the preparation and purification of the antigen may be related to different sensitivity and specificity. For this reason, serological tests are only recommended for surveillance studies, whereas direct methods should be used for food safety purposes.

Introduction

To date, the gold standard method for the detection of Trichinella is the digestion assay (OIE, 2009). However, there are several methods available for trichinellosis diagnosis, which are not recommended because of their lack of efficiency or reliability. In this sense, a diagnostic assay should be validated and may allow a repetitive measure. Although several serological tests have been developed, the Enzyme-Linked Immunosorbent Assay (ELISA) is considered as the test of choice based on economy, reliability, adaptability to good quality assurance practices, increasing body of validation data and good sensitivity and specificity when conducted under appropriate conditions (OIE, 2009).

ELISA represents a useful method for the rapid detection of specific antibodies in different body fluids, just as serum, plasma or meat juice. It is a useful tool for testing populations and is routinely used for surveillance programmes and outbreaks investigations. In this sense, antigen preparations have been developed to provide a high degree of specificity for Trichinella infection in swine (Gamble et al., 1988). Moreover, the Excretory-Secretory (ES) antigens from T. spiralis has been reported to be conserved in all species and genotypes of Trichinella (Ortega-Pierres et al., 1996), making feasible the detection of specific anti-Trichinella antibodies in pigs infected by any of the eight species of Trichinella.

For these reasons, two commercially available ELISA kits were compared with the artificial digestion method in order to determine the usefulness of each test for trichinellosis diagnosis. In our study ELISA test PrioCHECK® Trichinella Ab (Prionics) for Trichinella spp. showed positive results only in three animals, however, all the animals displayed negative results by the second ELISA test ID Screen® Trichinella Indirect (IDVet Innovative Diagnostics). In addition, all the sampled animals yielded negative results by the reference method of detection (magnetic stirrer method for pooled sample digestion; EC-2075/2005).

Material and Methods

A total of 709 Iberian pigs belonging to 79 free-range herds were randomly selected and sampled during 2008 and 2009. Sample size was assessed by the software Win Episcope version 2.0 on the basis of the number of samples required for a previous unknown prevalence (95% confidence level and 8% accepted error were assumed and confidence intervals of the prevalence were calculated).
Five to ten pigs per herd were randomly sampled. Blood samples were collected at the slaughterhouse into evacuated tubes, allowed to clot at room temperature and centrifuged at 1200 X g for 10-15 minutes at room temperature. The serum was harvested and frozen at - 70 °C until testing.

Sera samples were analyzed for the detection of specific antibodies against Trichinella spp. by means of two different ELISA kits, following manufacturer's instructions (PrioCHECK Trichinella Ab, Prionics; and, ID Screen® Trichinella Indirect, IDVet Innovative Diagnostics). The cut-off value used for discriminating between positive and negative serum samples was 15% and 50% respectively.

Magnetic stirrer method for pooled sample digestion was routinely performed on all the animals sampled following the regulation EC-2075/2005 for the detection of Trichinella spp.

Results

The ELISA PrioCHECK® Trichinella Ab (Prionics) allowed to detect specific anti-Trichinella spp. antibodies only in 3 out of 709 animals (0.42% CI95: 0.14-1.23) and 3 out of 79 herds (3.80% CI95: 0.18-7.42). However, no positive result was obtained when the ELISA test ID Screen® Trichinella Indirect (IDVet Innovative Diagnostics) was used. In addition, all the sampled animals displayed negative results for routine artificial digestion (Table 1).

Table 1. Number of positive free-range herds and pigs against each technique used for the detection of Trichinella spp.

	Techniques		
	PrioCHECK® Trichinella Ab (Prionics)	*ID Screen® Trichinella Indirect (IDVet Innovative Diagnostics)*	*Artificial digestion*
Herds (n=79)	3 (3.80)	0 (0.00)	0 (0.00)
Pigs (n=709)	3 (0.42)	0 (0.00)	0 (0.00)

Discussion

ELISA represents a useful rapid method to detect the presence of specific antibodies on serum, plasma or meat juice collected before or after slaughter. Trichinella infestation levels as low as one larva/100 g of tissue has been detected by ELISA in pigs (Gamble et al., 2004). However, although ELISA has shown a high sensitivity in the detection of specific anti-Trichinella spp. antibodies, false-negative results may be observed due to infected animals do not develop an antibody response until 3–5 weeks post infection (Gamble, 1996). In addition, ELISA may yield a low rate of false-positive results due to the specificity of ELISA for Trichinella infection is variable according to the type and quality of the antigen employed in each test (OIE, 2009).

ELISA tests are able to detect specific antibodies in serum, which may appear just after a contact with a microorganism, despite no efficient infestation. Thus, the positive results obtained with the first ELISA test (Prionics) in our study may be related either to a lack of specificity of the test or to a contact but not infestation with the parasite.

In our study, both ELISA kits were coated with Trichinella E/S antigens, but differences in the preparation and purification of the antigen may be related to different sensitivity and specificity (OIE, 2009). In addition, both tests presented different cut-off points. Whereas ELISA PrioCHECK® Trichinella Ab (Prionics), which yielded three positive animals and herds, had a cut-off < 15%, the ELISA test ID Screen® Trichinella Indirect (IDVet Innovative Diagnostics), which generated only

negative results, presented a cut-off < 50%. Therefore, the differences observed in the cut-off values for each test may be involved in the discrepancy observed in sensitivity and specificity of each test.

Conclusion

The differences observed between the different methods used in this study point to serological tests may be only recommended for surveillance studies, whereas direct methods should be used to individual carcass inspection, as recommended by the OIE (2009).

References

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