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Short communication

Serological diagnosis of *Taenia solium* in pigs: No measurable circulating antigens and antibody response following exposure to *Taenia saginata* oncospheres

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ABSTRACT

Taenia solium taeniasis/cysticercosis is a zoonosis included in the WHO's list of neglected tropical diseases. Accurate diagnostic tools for humans and pigs are needed to monitor intervention outcomes. Currently used diagnostic tools for porcine cysticercosis all have drawbacks. Serological tests are mainly confronted with problems of specificity. More specifically, circulating antigen detecting tests cross-react with *Taenia hydatigena* and the possibility of transient antigens as a result of aborted infections is suspected. Furthermore, the hypothesis has been raised that hatched ingested eggs of other *Taenia* species may lead to a transient antibody response or to the presence of circulating antigen detectable by serological tests used for porcine cysticercosis. Here we describe the results of a study that consisted of oral administration of *Taenia saginata* eggs to five piglets followed by serological testing during five weeks and necropsy aiming at studying possible cross reactions in serological tests used for porcine cysticercosis. The infectivity of the eggs was verified by in vitro hatching and by experimental infection of a calf. One piglet developed acute respiratory disease and died on day 6 post infection. The remaining four piglets did not show any clinical signs until euthanasia. None of the serum samples from four piglets collected between days 0 and 35 post infection gave a positive reaction in the B158/B60 Ag-ELISA and in a commercial Western blot for antibody detection. In conclusion, this study showed that experimental exposure of four pigs to *T. saginata* eggs did not result in positive serologies for *T. solium*. These results may help interpreting serological results in monitoring of *T. solium* control programmes.

1. Introduction

Taenia solium taeniasis/cysticercosis is a zoonosis of considerable public health and economic concern that mainly affects poor communities in low income countries. *T. solium* is on the list of neglected tropical diseases (NTD) of the WHO, which has set a goal of having validated strategies for control and elimination and scaled up interventions for this zoonosis by 2020 (WHO, 2016). Control programmes require accurate diagnostic tools for both humans and pigs to measure the impact of interventions. Various diagnostic tools are being used to detect porcine cysticercosis. Tongue palpation and carcass inspection are widely used but show poor sensitivities (Dorny et al., 2004). Serological assays, for detecting either specific antibodies or circulating antigens provide an alternative; however, the unpractical test formats, high cost and poor performances in terms of sensitivity and specificity limit their application. A common feature in antibody detection in porcine cysticercosis is that many seropositive pigs do not actually carry cysticerci (Gonzalez et al., 1994; Sciutto et al., 1998). False-positive/transient reactions in antibody tests for porcine cysticercosis can be due to exposure to *T. solium* eggs, which did not lead to the establishment of cysticerci or are the result of resolved infections or maternal antibodies. In antigen detecting tests cross-reactivity with *T. hydatigena* is also a major obstacle for the use of these tests in pigs in *T. solium* monitoring programmes in some areas (Nguyen et al., 2016). The possibility of transient antigens was raised following observations of short time antigen detection in sentinel pigs (Devleesschauwer et al., 2013) that could have been the result of an infection aborted during the early larval development. In addition, Lightowlers et al. (2016) argued that in the case of tests known to cross-react with other *Taenia* spp. the transient serologically antibody or antigen positives could be due to exposure to eggs of *T. hydatigena,* a non-zoonotic cestode that is highly prevalent in pigs in some *T. solium* endemic areas (Nguyen et al., 2016)

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and even other *Taenia* spp. for which pigs are not the natural intermediate host. Indeed, Lightowlers et al. (2016) raised the possibility that eggs of other *Taenia* species, such as *T. saginata* may hatch if ingested by pigs, followed by penetration of the oncospheres and a transient invasion of the tissues, possibly causing a transient antibody response or the presence of circulating antigen that could be detected by serological tests used for porcine cysticercosis.

Here we describe the results of a study that consisted of oral administration of *T. saginata* eggs to piglets followed by serological testing and necropsy aiming at studying possible cross reactions in serological tests used for porcine cysticercosis. A calf was infected with eggs from the same tapeworm to verify their infectivity.

2. Materials and methods

2.1. Study design

Five weaned 6 weeks old piglets of Belgian landrace breed were purchased from a commercial farm in Flanders, Belgium and transported to the experimental animal facility of the Veterinary Research Building, Faculty of Veterinary Medicine of Ghent University, Merelbeke, Belgium. They were housed together on an elevated slatted platform with a surface of 17 m^2, fed with commercial pelleted pig feed and given water ad libitum. On arrival they weighed 23 kg on average. They were bled 6 times from the anterior vena cava at weekly intervals from day 0 prior to administration of *T. saginata* eggs until euthanasia at day 35 (average weight 50 kg).

At the start of the study a 5 months old calf, purchased from a commercial farm was housed in an individual box on straw at the experimental animal facility of the Faculty of Veterinary Medicine of Ghent University. It was given hay, commercial feed and water ad libitum. The calf was bled 11 times from the jugular vein at weekly intervals from day 0 prior to administration of *T. saginata* eggs until euthanasia at day 70. The calf was kept until 10 weeks post infection for allowing cysticerci to reach full maturity and collect them for antigen production.

Blood from piglets and the calf was taken using 18G needles in vacutainer plain tubes. Serum was obtained from the blood samples and stored at −20 °C until analysis. The health of all animals was monitored daily.

2.2. T. saginata eggs and oral administration

Fresh gravid proglottids of *T. saginata* were obtained via a medical laboratory in Belgium from a human carrier following treatment. Species determination was done by the Polymerase Chain Reaction targeting a partial sequence of mitochondrial 12S DNA followed by Restriction Fragment Length Polymerase using restriction enzymes *Dde*I and HinfI, which generate specific restriction patterns for *T. saginata*, *T. solium* and *T. asiatica* (Rodriguez-Hidalgo et al., 2002; Somers et al., 2007). After washing of the proglottids in buffered phosphate saline (PBS) the eggs were manually extracted and counted under a light microscope at X100 magnification. The eggs were stored at 4 °C in PBS to which Antibiotic − Antimycotic (100X) (Gibco 15240096) was added (penicillin 100 Units/ml, streptomycin 100 µg/ml and Amphotericin B 0.25 ug/ml) and used for infection two months after collection. Prior to administration, hatching of the eggs was measured by the sodium hypochlorite method (Wang et al., 1997). The doses for administration were prepared in PBS at a concentration of approximately 1000 eggs/ml. Infection was done orally using a disposable syringe, 5000 eggs in piglets, and 30,000 eggs in the calf.

2.3. Euthanasia and necropsy

Euthanasia in piglets was performed by electrical stunning (Stunning transformer Type EC-2-1/MRE), following directive 2009/1099/EC. The calf was euthanized by intravenous injection of T61® (MSD Animal Health). Euthanasia was followed by exsanguination from the jugular vein. Carcasses and heads were skinned and the muscles separated from the bones. All skeletal muscles, heart, tongue, diaphragm, spleen, kidneys, lungs, liver, eyes and brain were dissected on the day of euthanasia for pigs, and over a period of 4 days for the calf. Slices of maximum 0.5 cm thick were made for inspection of cysticerci that were enumerated and classified as viable, degenerated or calcified (Phiri et al., 2006).

2.4. Serological analysis

The B158/B60 enzyme-linked immunosorbent assay (Ag–ELISA) was performed on serum samples from piglets and the calf to detect circulating *Taenia* antigens (Dorny et al., 2000; Dorny et al., 2004). The status of the test samples was determined by comparing their optical densities to those of 8 negative control sera (from pigs or cattle) at a probability of $p < 0.001$ (Sokal and Rohlf, 1981). Two positive control sera were run on each plate as a supplementary quality check. Additionally, in order to detect antibodies in the piglet samples, a cysticercosis Western blot (LDBIO Diagnostics, Lyon, France) was used according to the manufacturer's instructions, except for the conjugate and the positive control that were substituted by a goat anti-pig IgG polyclonal serum conjugated with alkaline phosphatase diluted at 1/5000 (ab 10128, Abcam) and a serum sample from a pig harboring viable *T. solium* cysticerci, respectively. The optimal concentration of the conjugate was selected following serial dilutions. The presence of a minimum of two well-defined bands among the five described bands, P6-8, P12, P23-26, P39, and P50-55, is indicative of cysticercosis (LDBIO Diagnostics, 2012). This test was validated on porcine serum samples by Porphyre et al. (2016).

2.5. Ethical clearance

All procedures employed in this study were approved by the Ghent University Ethics Committee of the Faculties of Veterinary Medicine and Bioscience Engineering.

3. Results

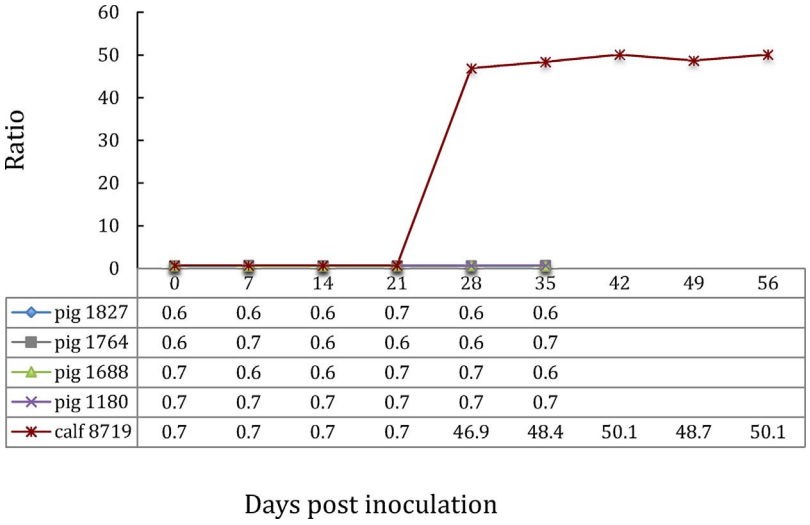
One piglet developed acute respiratory disease and died on day 6. The remaining four piglets and the calf did not show any clinical signs until euthanasia. More than 80% of the *T. saginata* eggs hatched in a hypochlorite solution when checked prior to administration to the piglets and the calf.

None of the serum samples from four piglets collected at 6 sampling days between days 0 and 35 gave a positive reaction in the Ag-ELISA: the optical densities were similar for all piglets on all sampling days and remained far below the cut off (Fig. 1). Similarly, in the Western blot all pig serum samples remained negative on all sampling dates while the positive control gave a characteristic banding pattern (data not shown). At necropsy, no cysticerci were found in any of the piglets.

The Ag-ELISA became positive in the calf at day 28 post infection; optical densities remained elevated until necropsy (Fig. 1). In the calf, a total of 3072 cysticerci were counted at necropsy consisting of 2684 viable and 388 degenerated cysticerci.

4. Discussion

In this study, no antibody response nor circulating antigens were measured by a commercial Western blot for *T. solium* diagnosis and the B158/B60 Ag-ELISA, respectively in piglets orally inoculated with 5000 *T. saginata* eggs. The infectivity of the inoculum was confirmed by the successful experimental infection using the same batch of eggs in a calf. These results suggest that exposure of piglets to eggs of *T. saginata*, a species that does not cause natural infection in pigs, does not result in



	0	7	14	21	28	35	42	49	56
pig 1827	0.6	0.6	0.6	0.7	0.6	0.6			
pig 1764	0.6	0.7	0.6	0.6	0.6	0.7			
pig 1688	0.7	0.6	0.6	0.7	0.7	0.6			
pig 1180	0.7	0.7	0.7	0.7	0.7	0.7			
calf 8719	0.7	0.7	0.7	0.7	46.9	48.4	50.1	48.7	50.1

Fig. 1. B158/B60 Ag-ELISA results of 4 pigs and one calf experimentally inoculated with *Taenia saginata* eggs. Results are expressed in Ratio = OD sample/cut off. A result is considered positive at Ratio > 1.

transient antibody and antigen responses, in contrast to the hypothesis of Lightowlers et al. (2016). These authors raised the possibility that oncospheres released from eggs of a heterologous *Taenia* spp. may pass the gut barrier and transiently establish before being eliminated by the immune system, possibly resulting in positive serological tests. In piglets experimentally infected with *T. solium*, circulating antigens may be detected as early as two weeks post-infection, many weeks before the cysticerci have reached maturity. Antibodies appear within 3–4 weeks after infection (Deckers et al., 2008). Therefore, the 5 weeks monitoring period following egg exposure in our study should have allowed the detection of a possible seroconversion or the presence of circulating antigens.

We acknowledge a number of weaknesses in the present study, i.e. the low number of animals, the use of a commercial antibody test developed for the diagnosis of human cysticercosis and the fact that we worked in controlled experimental conditions on well-fed pigs that do not compare with common field situations in *T. solium* endemic regions that may impair the pig's immune system and make them more susceptible to infections. These points should be addressed in further work that should also look at other infection doses and in trickle infections.

In conclusion, this study has shown that exposure of pigs to *T. saginata* eggs under experimental conditions does not result in positive serologies for *T. solium.* These results may help interpreting serological results in monitoring of *T. solium* control programmes.

Conflicts of interest

None

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