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Taenia hydatigena in pigs in Burkina Faso: a cross-sectional abattoir study

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Abstract

Taenia hydatigena is a non-zoonotic cestode that has canines as definitive hosts and ruminants and pigs as intermediate hosts. In pigs, its presence causes cross-reactivity in serological testing for *Taenia solium* cysticercosis. Therefore, knowledge on the occurrence of *T. hydatigena* is paramount for validly estimating the seroprevalence of *T. solium* cysticercosis in pigs. In a cross-sectional abattoir study, we estimated the prevalence of *T. hydatigena* in pigs slaughtered in Koudougou, Burkina Faso. Carcasses of 452 pigs were examined by investigators for perceived and suspected *T. hydatigena* cysticercus lesions in the abdominal cavity or on the surface of abdominal organs. Routine meat inspection was performed by local inspectors to identify *T. solium* cysticerci. All lesions were subjected to PCR-RFLP analysis in order to differentiate *Taenia* spp. Additionally, individual blood samples were examined for the presence of circulating cysticercus antigens using the B158/B60 Ag-ELISA. Perceived *T. hydatigena* cysticerci were found in 13 pigs, whereas meat inspectors found seven carcasses infected with *T. solium* cysticerci. All were confirmed by molecular analysis. Of pigs with other suspected lesions, mostly located in the liver, 27 and six were found to harbour *T. hydatigena* and *T. solium* cysticerci, respectively. Overall, 8.8% of pigs (40/452) were found infected with *T. hydatigena* and 2.9%

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(13/452) with *T. solium*. Of these positive pigs, one was found infected with both *Taenia* spp. (0.2%, 1/452). Blood samples of 48.5% of pigs (219/452) were positive in the Ag-ELISA. Pigs with confirmed cysts of *T. hydatigena* and *T. solium* had a positive Ag-ELISA result in 57.5% (23/40) and 61.5% (8/13) of cases, respectively. The observed *T. hydatigena* prevalence in this study is relatively high in comparison to other studies in Africa. Estimates of the occurrence of active porcine *T. solium* infection using the B158/B60 Ag-ELISA should therefore be adjusted for the presence of *T. hydatigena.* The low level of *T. solium* infection detected upon meat inspection in this study is likely an underestimation of the true prevalence since routine meat inspection shows poor sensitivity and pigs perceived to be infected based on tongue palpation are rarely sent to official abattoirs.

Keywords

Taenia hydatigena; *Taenia solium*; cysticercosis; pigs; Burkina Faso

1. Introduction

Pigs may act as the intermediate hosts of *Taenia hydatigena*, a non-zoonotic cestode that has canines as definitive hosts. Infection with this parasite rarely leads to clinical signs in pigs, but is causing cross-reactions in serological tests developed for the detection of *Taenia solium* cysticercosis (Dorny et al., 2004a). Worldwide, the occurrence of *T. hydatigena* in pigs varies widely. In Asia, studies have reported prevalences as high as 25.7% in China (Yin et al., 2013), and 22.4% in Laos (Conlan et al., 2012). In contrast, the reported prevalence in Africa seems to be much lower, ranging from 1.4% to 6.7% (Ngowi et al., 2004; Permin et al., 1999).

Pigs are also the intermediate hosts of *T. solium* that has humans as definitive hosts. Whereas humans are not susceptible to infection with *T. hydatigena*, they can acquire *T. solium* through the consumption of undercooked infected pork, leading to the development of an intestinal adult tapeworm infection (taeniosis). In addition, ingestion of tapeworm eggs by humans and pigs can lead to cysticercosis. In humans, cysticerci can establish in the central nervous system causing a condition called neurocysticercosis (NCC). NCC can be accompanied by severe signs and symptoms such as epilepsy and chronic headaches (Carabin et al., 2011). Considering its zoonotic character, it is important to monitor the presence of *T. solium* in humans and pigs as part of disease control and surveillance (WHO, 2016).

In pigs, various diagnostic tools have been described to detect *T. solium* cysticercosis (Lightowlers et al., 2016). Tongue palpation and carcass inspection are widely used for ante and post-mortem diagnosis, respectively. However, these tools show poor sensitivities of 16.1% (95% confidence interval (95%CI): 5-34) and 38.7% (95%CI: 22-58), respectively (Dorny et al., 2004b). Serological assays, whether aimed at detection of antigens (Ag) or antibodies (Ab), provide a better alternative as they show higher test performances.

The B158/B60 Ag-ELISA is a serological assay that detects circulating antigens of *Taenia* spp., of which the presence is an indication of infection with viable cysticerci. The test has a

reported sensitivity and specificity to detect both viable and dead *T. solium* cysticerci in pigs of 64.5% (95% CI: 45-81) and 91.2% (95%CI: 76-98), respectively (Dorny et al., 2004b). However, cross reactivity exists in this test between *T. solium* and other *Taenia* spp., such as *T. hydatigena*, due to its genus but not species-specific nature (Dorny et al., 2004a). In Burkina Faso, a large on-going community randomized-control trial (EFECAB) aims to estimate the impact of an educational intervention on human and porcine cysticercosis (Carabin et al., 2015). In this trial, the B158/B60 Ag-ELISA is used as a diagnostic tool to estimate levels of porcine cysticercosis. Currently, no studies have investigated the presence of *T. hydatigena* in pigs in Burkina Faso, although knowledge on the occurrence of this parasite is essential to interpret levels of *T. solium* cysticercosis estimated through the B158/B60 Ag-ELISA. Therefore, the aim of the present study was to estimate the prevalence of *T. hydatigena* among pigs slaughtered at the Koudougou abattoir, Burkina Faso.

2. Materials & methods

2.1. Study area & sampling strategy

A cross-sectional abattoir study was conducted in Koudougou, a small city located about 100 km west of the capital Ouagadougou. Pigs slaughtered in the Koudougou abattoir typically originate from Koudougou and nearby villages. In these areas, traditional pig management is practiced, meaning pigs are left roaming to scavenge for their food during the dry season. In 2010, the pig population in the province of Boulkiemdé, where Koudougou is located, was estimated at 191,438, the largest in the country (Ministère des Ressources Animales, 2010). Yet, not all households in the region consume pork or own a pig (Ganaba et al., 2011).

The planned sample size for the current study was 384, assuming a large population size with unknown *T. hydatigena* prevalence and setting the required confidence level at 95% with ±5% precision (Cochran, 1977). Ultimately, 452 pigs were randomly sampled at the Koudougou abattoir in March and April 2015.

Upon slaughter, a jugular blood sample was collected in an additive-free blood tube from each pig. The age, sex and origin of sampled pigs were noted. Blood samples were allowed to clot, after which serum was obtained. Serum aliquots were stored at −20°C until further analysis.

One researcher subjected pigs to detailed post-mortem inspection for the presence of *T. hydatigena* cysticerci in the abdominal cavity and on the surface of the liver and other abdominal organs. Samples were categorized as “perceived *T. hydatigena* cysts” (i.e., large loose-hanging cysts attached to an organ surface) or “suspected lesions” (i.e., other smaller lesions). Two local meat inspectors conducted their routine examination according to national regulations (Conseils des Ministres, 1989). The examination involved detailed inspection of all abdominal and thoracic organs (including incision of the heart and lymph nodes), as well as inspection of the directly visible muscular surfaces, in particular of the thigh, psoas, intercostal muscles, abdominal wall, pillars of the diaphragm, heart, tongue and larynx (Conseil des Ministres, 1989). Lesions deemed positive for *T. solium* by meat

inspectors were defined as “lesions identified as *T. solium* upon meat inspection”. All lesions were collected in 70% ethanol and kept at ambient temperature until further analysis.

2.2. Laboratory analyses

Molecular methods were used to identify and differentiate *Taenia* spp. in sampled lesions. First, genomic DNA was extracted using the DNeasyBlood and Tissue Extraction Kit according to the manufacturers’ instructions (QIAGEN, Hilden, Germany). PCR was used to amplify a mitochondrial 12s rDNA gene fragment with the primer set ITM TnR-TaenF (Rodriguez-Hidalgo et al., 2002). Afterwards, restriction fragment length polymorphism (RFLP) was used to differentiate the *Taenia* spp. The restriction enzymes *Dde*I and *Hinf*I were used to identify *T. solium* (Rodriguez-Hidalgo et al., 2002) and *Hpa*I was used to identify *T. hydatigena* (Devleesschauwer et al., 2013). In case the observed pattern was equivocal, sequencing was performed on the PCR product to obtain full confirmation of the species in sampled lesions. Sequencing was performed at the VIB Genetic Service Facility (University of Antwerp, Belgium). BioEdit was used to edit and align obtained sequences (Hall, 1999) and BLAST was performed on NCBI.

A monoclonal antibody-based B158/B60 Ag-ELISA (Brandt et al., 1992; Dorny et al., 2004b) was used to detect the presence of circulating cysticercus antigens in serum. The Ag-ELISA was performed as described by Dorny et al. (2000), with slight modifications described by Dorny et al (2004b). Briefly, the monoclonal antibody B158C11A10, diluted at 5 μg/ml was coated on the wells of Nunc Maxisorp microtitre plates. After washing and blocking of the wells, serum samples pre-treated with 5% trichloro acetic acid were added. Next, after washing, biotinylated monoclonal antibody B60H8A4 diluted at 1.25 μg/ml in phosphate buffered saline-Tween 20 (PBS-T20) + 1% new born calf serum (NBCS) was used to detect the circulating cysticercus antigens. Streptavidin-horseradish peroxidase (Jackson Immunoresearch Lab, Inc.) diluted at 1/10,000 in PBS-T20+1% NBCS was used as the conjugate. Incubations were done at 37°C on a shaking plate and followed for each step by washing five times with PBS-T20 using an automated ELISA washer. Finally, ortho-phenylenediamine (Dako, Glostrup, Denmark) was used as the substrate. After 15 minutes of incubation in the dark, the reaction was stopped with 50 μl 4 N H_2SO_4. Optical densities in the wells were read using a spectrophotometer (Titertek Multiskan EIA reader, Vienna, Virginia, USA) at 492 nm and a reference at 655 nm.

Serum samples from two pigs confirmed to be heavily infected were used as positive controls. The cut-off was calculated for each plate based on the optical density (OD) of eight negative reference sera based on a variation of the student *t*-test distribution at a probability of $p = 0.001$ (Sokal and Rohlf, 1991). The OD of each sample was divided by the cut-off to obtain a ratio. Ag-ELISA ratios of positive pigs were classified as follows: low: $1 \leq \text{ratio} < 2$; medium: $2 \leq \text{ratio} < 5$; high: $5 \leq \text{ratio} < 10$; very high: $\text{ratio} \geq 10$ (Praet et al., 2010).

2.3. Statistical analyses

All collected data were entered into Excel (Microsoft Office Excel 2010). Descriptive statistical analyses (i.e., calculation of percentages) were conducted using R, version 3.2.4 (R Core Team, 2016).

3. Results

A total of 452 pigs were inspected at the Koudougou abattoir, Burkina Faso. The majority of these pigs originated from Koudougou (311/452, 68.8%), while the others came from 12 surrounding villages. Half of the sampled pigs were female (225/452, 49.8%) and most were younger than two years (405/452, 89.6%). A total of 58 suspected lesions were collected among 57 of the 452 inspected pigs (Table 1). Thirteen of these lesions were perceived to be cysticerci of *T. hydatigena*, whereas local meat inspectors identified seven carcasses as being infected with *T. solium* cysticerci. Suspected lesions without clear diagnosis were found in livers of an additional 37 pigs (of which one pig also had another cyst perceived to be *T. hydatigena*). One pig had a suspected lesion in the spleen.

Molecular analysis of sampled lesions confirmed the diagnosis of all perceived *T. hydatigena* cysts ($n = 13$) as well as the identification of *T. solium* positive carcasses by local meat inspectors ($n = 7$). Suspected liver lesions of 27 pigs were identified as *T. hydatigena*, and in six pigs, liver lesions were identified as *T. solium*. No *Taenia* spp. could be identified in four pigs with suspected liver lesions or in the pig with the suspected spleen sample. Overall, based on molecular analysis of sampled lesions, 8.8% of pigs (40/452) were found infected with *T. hydatigena* and 2.9% (13/452) with *T. solium*. One of these positive pigs was found infected with both *Taenia* spp. (overall 0.2%, 1/452).

The Ag-ELISA identified 48.5% of pigs (219/452) as positive for circulating cysticercus *Taenia* spp. antigens (Table 2). Cysticerci of *T. hydatigena* or *T. solium* were identified in 13.7% of sero-positive pigs (30/219), with *T. hydatigena* being identified in 10.5% (23/219). Conversely, the Ag-ELISA was negative in 42.3% (22/52) of pigs found to harbour *T. solium* and/or *T. hydatigena* cysts. Overall, *T. hydatigena* and *T. solium* confirmed cases were Ag-ELISA positive in 23 out of 40 (57.5%) and eight out of 13 (61.5%) pigs, respectively.

Approximately half of *T. hydatigena* positive pigs (19/40) had an Ag-ELISA ratio below 2 (Table 3), whereas almost all of the remaining pigs had a very high ratio (≥10) (19/40). All pigs with samples perceived to be obvious *T. hydatigena* cysts ($n = 13$) had very high ratios.

4. Discussion

This is the first study reporting the prevalence of *T. hydatigena* in pigs in Burkina Faso and the first study on *T. hydatigena* prevalence in pigs to include molecular confirmation. The prevalence of *T. hydatigena* found in this study is relatively high (8.8%) in comparison to other African studies, where the reported prevalence in pigs ranged between 1.4% (Tanzania: Ngowi et al., 2004) and 6.6 to 6.7% (6.6% (16/243) in Tanzania (Braae et al., 2015), 6.7% (4/60) in Ghana (Permin et al., 1999)) (reviewed by Nguyen et al., 2016). Pigs in those studies were sampled in small abattoirs, in villages or in both, and prevalence estimates were based on post-mortem carcass inspection only.

The proportion of *T. solium* infected carcasses identified through meat inspection (1.5%, 7/452) in the current study was higher than the national prevalence of 0.6% reported by public authorities in 1997 (Coulibaly and Yameogo, 2000). The reported proportions of *T.*

solium infected carcasses identified upon meat inspection in other West African countries varied widely from 1.2% (Senegal) to 20.5% (Nigeria) (Zoli et al., 2003). Such a large range in estimates may be explained by variation in meat inspection regulations between countries and disparate adherence to inspection regulations, even within countries (WHO, 2015; Zoli et al., 2003). Furthermore, it is known that routine meat inspection is largely insufficient to detect all *T. solium* positive carcasses, especially in the case of light infections, due to its low sensitivity (Boa et al., 2002; Dorny et al., 2004b; Gonzalez et al., 1990). For instance, in a study in Zambia, 31 out of 65 pigs were found to harbour *T. solium* cysts upon complete dissection whereas only 12 were identified as positive upon meat inspection (Dorny et al., 2004b). Hence, the proportion of *T. solium* positive pigs found through meat inspection in Koudougou cannot be considered as a valid estimate of the prevalence of porcine cysticercosis in the region.

In the current study, 48.5% of pigs slaughtered at the Koudougou abattoir were positive in the Ag-ELISA, indicating a high level of active *T. solium* infections among pigs slaughtered in an official abattoir in this area. In two villages in the centre of Burkina Faso, where pig breeding and pork consumption are very common, Ganaba et al. (2011) found that 32.5% and 39.6% of the pigs were positive in the Ag-ELISA, respectively. In the Democratic Republic of the Congo, 38.4% and 41.2% of pigs were found positive in the Ag-ELISA in markets and in villages, respectively (Praet et al., 2010). In free-ranging pigs in Zambia, a sero-prevalence of 23.3% was observed (Sikasunge et al., 2008), whereas at a local slaughter place, 57.1% of sampled pigs were found positive in the Ag-ELISA (Dorny et al., 2004b). The level of Ag-ELISA positive pigs was thus high in all these countries, suggesting a widespread presence of porcine cysticercosis. Tongue palpation is often performed by pig owners to screen their own animals (Gonzalez et al., 1990), as it will detect pigs heavily infected with *T. solium* (Dorny et al., 2004b). In case an infected pig is identified, it is commonly home slaughtered or sold at a local market, and thus *de facto* excluded from the official pig supply chain (Morales et al., 2006; Ngowi et al., 2004; Praet et al., 2010). If this practice is also common in Burkina Faso, the true proportion of *T. solium* positive pigs in the area may even be higher.

A false negative Ag-ELISA result was observed in five out of 13 confirmed *T. solium* positive pigs in the current study. As the Ag-ELISA is designed to detect active infections, and has a reported sensitivity for overall infections (i.e., for both viable and dead cysticerci) of around 64.5% (Dorny et al., 2004b), our data confirm that it will even underestimate prevalence of *T. solium* at the slaughterhouse level. Estimates of *T. solium* prevalence based on Ag-ELISA results should therefore be adjusted for its test performance characteristics. On the other hand, *T. hydatigena* is known to cross-react in the Ag-ELISA (Dorny et al., 2004a). Considering the relatively high prevalence of *T. hydatigena* in this study, it is suggested that estimates of current *T. solium* infection prevalence based on Ag-ELISA results in this region should equally be adjusted for the presence of *T. hydatigena*.

Almost 70% of the pigs sampled in this study originated from the town of Koudougou. Sampling of pigs in the abattoir of Koudougou was preferred over sampling in villages due to practical constraints. However, this raises the question of whether it is possible to generalise the estimated prevalence of *T. hydatigena* found in this study to all study villages

included in EFECAB. Nonetheless, livestock management practices are not believed to vary much between Koudougou and villages in the area, suggesting that the estimated prevalence of *T. hydatigena* may actually be a reasonable estimate to adjust for the validity of the Ag-ELISA in this region.

Conclusions

In the present study, a high proportion of *T. hydatigena* infections was found among pigs slaughtered at the Koudougou abattoir in Burkina Faso, relative to what had been previously reported in other African countries. This finding has important consequences for the interpretation of the results of the B158/B60 Ag-ELISA when used in studies in the region. Hence, estimates of the occurrence of active porcine *T. solium* infection in this area should be adjusted for the presence of *T. hydatigena*. Nevertheless, the inherent underestimation of the *T. solium* prevalence in pigs by the B158/B60 should also be taken into account.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Highlights

- *Taenia hydatigena*, a non-zoonotic cestode, can have pigs as intermediate hosts
- It causes cross-reactivity in serological tests for active *T. solium* cysticercosis
- In Koudougou, Burkina Faso, a prevalence of 8.8% was found at the abattoir
- Estimates of active porcine cysticercosis should be adjusted for *T. hydatigena*

Table 1

Number of pigs with suspected *Taenia hydatigena* and *Taenia solium* lesions sampled for molecular confirmation in a cross-sectional study conducted in March-April 2015 at the Koudougou abattoir, Burkina Faso (n = 452).

	Molecularly confirmed species			
Sample	**Total**	***T. hydatigena***	***T. solium***	**None**
Perceived *T. hydatigena* cysts	13	13	0	0
Lesions identified as *T. solium* upon meat inspection	7	0	7	0
Suspected liver lesions	37	27	6	4
Suspected spleen lesions	1	0	0	1
Total	58	40	13	5

Table 2

Proportion of Ag-ELISA and *Taenia hydatigena* negative and positive pigs sampled at the Koudougou abattoir, Burkina Faso ($n = 452$).

Ag-ELISA serum	*T. hydatigena*	*n*	Percentage (%)
Negative	Negative	216	47.8
Negative	Positive	17	3.8
Positive	Negative	196	43.4
Positive	Positive	23[a]	5.1

[a]This group included one pig with a confirmed *T. solium* cyst as well

Table 3

Ag-ELISA ratio in *Taenia hydatigena* positive pigs sampled at the Koudougou abattoir, Burkina Faso (*n* = 40).

Ratio[a]	*n*	Percentage (%)
Low	19	47.5
Medium	1	2.5
High	1	2.5
Very high	19[b,c]	47.5

[a] Classes based on Ag-ELISA ratio: Low: 1 ≤ ratio < 2, Medium: 2 ≤ ratio < 5, , High: 5 ≤ ratio < 10, Very high: ratio ≥ 10 (Praet et al., 2010)

[b] This class included all pigs with cysts perceived to be obvious *T. hydatigena* cysts (*n* = 13) upon post-mortem examination

[c] This group included one pig with a confirmed *T. solium* cyst as well