Pig-farming systems and porcine cysticercosis in the north of Cameroon

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Abstract

A survey was conducted in 150 households owning 1756 pigs in the rural areas of Mayo-Danay division in the north of Cameroon. A questionnaire survey was carried out to collect information on the pig-farming system and to identify potential risk factors for *Taenia solium* cysticercosis infection in pigs. Blood samples were collected from 398 pigs with the aim of estimating the seroprevalence of *T. solium* cysticercosis. The results showed that 90.7% of the pigs are free roaming during the dry season and that 42.7% of households keeping pigs in the rural areas have no latrine facility. Seventy-six per cent of the interviewed pig owners confirmed that members of the household used open-field defecation. Enzyme-linked immunosorbent assay (ELISA) for antigen and antibody detection showed an apparent prevalence of cysticercosis of 24.6% and 32.2%, respectively. A Bayesian approach, using the conditional dependence between the two diagnostic tests, indicated that the true seroprevalence of cysticercosis in Mayo-Danay was 26.6%. Binary logistic regression analysis indicated that a lack of knowledge of the taeniasis–cysticercosis complex and the absence of a pig pen in the household were associated with pig cysticercosis.

Introduction

Taenia solium cysticercosis is a severe zoonotic disease, which causes serious public health problems and provokes economic losses in the areas where it is endemic, particularly in developing countries (Zoli *et al.*, 2003; Carabin *et al.*, 2006; Praet *et al.*, 2009). The important findings concerning the control of *T. solium* cysticercosis during the past two decades were reviewed by Pawlowski *et al.* (2005). One of the most important conclusions is that tapeworm carriers and human and porcine cysticercosis cases cluster in endemic foci. The risk factors for human cysticercosis are frequent consumption of pork, poor hygiene and having a history of taeniasis (Flisser & Gyorkos, 2007). Although the

presence of free-roaming pigs in rural areas of developing countries was identified as an important risk factor for swine cysticercosis (Sikasunge *et al.*, 2007), this is not the only way for pigs to get access to human faeces. Direct defecation of pig keepers in pig pens has been reported in the west of Cameroon (Zoli *et al.*, 1987; Shey-Njila *et al.*, 2003). The Mayo-Danay division is the most important region for pig breeding in northern Cameroon. In this part of the country, pigs are kept by non-Muslim people who interact with an important Muslim community. Pig husbandry is traditional and involves mainly local breeds which receive some domestic waste and little feed supplement (Njoya *et al.*, 1997; Koussou, 1999). Limited data from earlier studies in northern Cameroon suggested hyperendemicity of human and porcine cysticercosis (Awa *et al.*, 1999, Assana *et al.*, 2001), but details are not known concerning the factors that predispose to the infection of pigs. The main objective of this study was to

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update data on swine cysticercosis and identify risk factors for this zoonosis in the Mayo-Danay division.

Materials and methods

Study area

The Mayo-Danay division is located in the far northern province of Cameroon (fig. 1). The annual average rainfall varies from 600 to 800 mm and the wet season lasts 5 months – from May to October – while little or no rain falls during the rest of the year. Temperatures vary from maximum of around 45°C to a minimum of 20°C. The total population is about 600,000 inhabitants. There are four predominant ethnic groups: the Massa, the Toupouri, the Mousgoum and the Moussey, who are largely animistic or Christian and primarily involved in crop and livestock production. Livestock activities include keeping of cattle, sheep, goats, poultry and pigs. Pig raising is possible because most of the population is non-Muslim.

Survey design and data collection

A survey was conducted in Mayo-Danay division between June 2007 and April 2008. A one-visit, multiple-objects survey was carried out to collect information on the pig-farming system. One hundred and fifty households, keeping 1756 pigs, were randomly sampled out of the registries kept by the authorities in the major area of pig farming. These rural areas were identified following the preliminary study carried out by Assana *et al.* (2001). Three hundred and ninety-eight (398) blood samples from pigs were collected in these surveyed households. A preliminary study had shown that the seroprevalence of pig

Fig. 1. Map showing the study area (Mayo-Danay division, grey shading) in the north of Cameroon (all other Cameroon districts shown as white areas).

cysticercosis in Mayo-Danay was 39.8% (Assana *et al.*, 2001). Assuming that a maximum sample size of 384 is required at the prevalence level of 50% (Sikasunge *et al.*, 2008), at least 368 pigs were needed in this study according to the formula $n = Z^2 PQ/L^2$, where n is the required sample, Z is the Z score for confidence, P is the known prevalence, $Q = (1 - P)$ and L is the error estimation at 95% confidence (Martin *et al.*, 1987). Therefore, a sample size of 398 pigs is easily sufficient to estimate the prevalence of pig cysticercosis in Mayo-Danay division.

Serology

Serum samples

Serum samples collected from pigs during the survey were stored at −20°C until tested. Two positive serum samples from naturally infected pigs and eight negative serum samples from pigs from a local commercial pig farm without any history of cysticercosis were included as reference control sera in the enzyme-linked immuno-sorbent assay (ELISA).

Enzyme-linked immunosorbent assay for the detection of circulating T. solium antigens (Ag-ELISA)

The Ag-ELISA was performed as described by Brandt *et al.* (1992) and modified by Dorny *et al.* (2004). Briefly, the sera were pre-treated using trichloroacetic acid (TCA) and used in ELISA at a final dilution of 1/4. The monoclonal B158 was diluted at 5 μg/ml in carbonate buffer (0.06 M, pH 9.6) for coating the ELISA plate and the biotinylated monoclonal antibody (MoAb) B60 (1.25 μg/ml in phosphate-buffered saline containing 0.05% Tween 20 (PBS-T20)/1% newborn calf serum (NBCS)) was included as detector antibody. The incubation was carried out at 37°C on a shaker for 30 min, for the coating of the first MoAb, and for 15 min for all subsequent steps. The chromogen/substrate solution, consisting of orthophenylene diamine (Dako, Glostrup, Denmark) and H_2O_2 was added and incubated without shaking between 30 and 33°C for 15 min. To arrest the reaction, 50 μl of 4 N H_2SO_4 was added to each well. The plates were read using an ELISA reader (Multiskan RC, MTX Lab Systems, Vienna, Virginia, USA) at 492 nm.

Enzyme-linked immunosorbent assay for the detection of antibodies against T. solium cysticerci (Ab-ELISA)

The ELISA for the detection of antibodies against *T. solium* cysticerci (Ab-ELISA) was based on a 14 kDa antigen (F3 antigen) purified from *T. solium* cyst fluid by a two-step chromatography, as described by Assana *et al.* (2007). The antigen (F3) was diluted at 1 μg/ml in a carbonate buffer (0.06 M, pH 9.6) for coating (1 h at 37°C and overnight at 4°C). PBS-T20 was used for washing between steps. Serum samples and conjugate were blocked and incubated on a shaking plate for 1 h at 37°C. PBS−T20/NBCS was used for blocking. Serum samples were diluted at 1/300. A rabbit anti-pig IgG peroxidase conjugate (Sigma, St. Louis, Missouri, USA) was used at a dilution of 1/30,000. The wells were washed three times, and the final steps were executed as described for the Ag-ELISA.
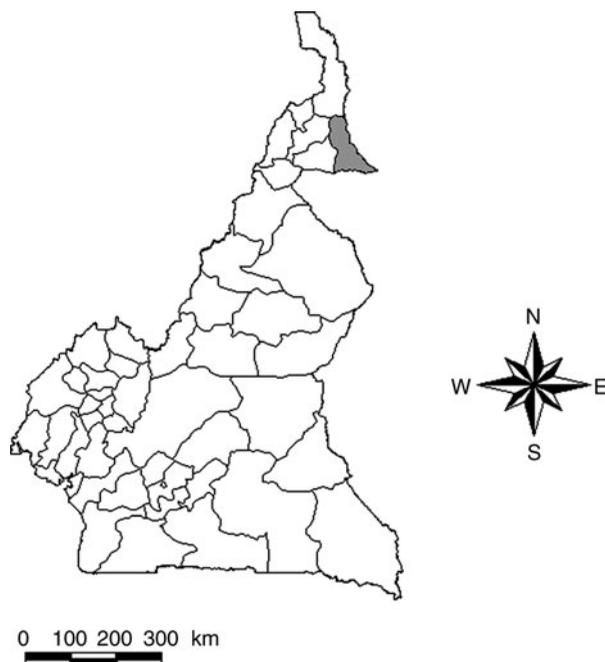
For both Ag- and Ab-ELISA, the optical density (OD) of each serum sample was compared with a series of reference negative serum samples ($n = 8$) at a probability level of $P = 0.001$ to determine the cut-off, using a modified Student's t-test (Sokal & Rohlf, 1981).

Statistical analysis

A Bayesian analysis was used to estimate the true prevalence of cysticercosis in the Mayo-Danay division and the sensitivity (Se) and specificity (Sp) of both Ag-ELISA and Ab-ELISA tests using a multinomial model as described by Krecek *et al.* (2008). Briefly the results from Ag-ELISA and Ab-ELISA were combined together in a Bayesian model and run in Wingbugs 1.4 (Spiegelhalter *et al.*, 2003). The prior information was obtained from previous studies on diagnosis of cysticercosis (Pouedet *et al.*, 2002; Dorny *et al.*, 2004; Assana *et al.*, 2007). The sensitivity and specificity of the Ag-ELISA were constrained to intervals (0.8, 1) and (0.9, 1), respectively, whereas the sensitivity and specificity of the Ab-ELISA were constrained to intervals (0.4, 1) and (0.5, 1). The validation of the model was based on the number of parameters (P_D), the Deviance Information Criterion (DIC) values from posterior mean of the multinomial probabilities (DIC_Pr) and from posterior mean of the parameter of the model using parent nodes (DIC_P) and on the Bayesian-p values (Bayesp) as described by Berkvens *et al.* (2006). To identify risk factors for pig cysticercosis, logistic regression was performed as described by Prasad *et al.* (2007). A pig that had a positive result in the Ag- or Ab-ELISA was considered as an infected pig in the analysis of risk factors.

Results

Pig-farming systems and sanitary facilities

Most of the pig farmers (90.7%) in the Mayo-Danay division kept pigs in a free-roaming system during the dry season. In the rainy season, pigs were confined in a small pig pen. From the 150 farmers interviewed, only 4.9% practised permanent indoor pig raising, whereas 13.7% allowed the pigs to roam freely during the rainy season (since there is sometimes overlapping of various systems, the total figure exceeds 100%). The survey showed that 16% of the households did not have a pig pen. Furthermore, it revealed that 42.7% of the house-holds keeping pigs had no latrine facilities and that 76.0% of the pig farmers declared that the members of their family used open-field defecation.

Serology

The overall seroprevalence of pig cysticercosis was 24.6 and 32.2% for Ag-ELISA and Ab-ELISA, respectively.

Of the total of 150 households surveyed, 56.0% and 57.3% of them had at least one pig positive for porcine cysticercosis in Ag-ELISA and Ab-ELISA, respectively.

Bayesian analysis

There was a good correlation between the results obtained by Ag-ELISA and Ab-ELISA. Two hundred and twenty-eight out of 398 sera were negative in both Ag- and Ab-ELISA, whereas 56 out of 398 sera were positive in both tests. The estimated true prevalence using Bayesian analysis was 26.6% (95% CI: 15.6–31.0%). Forty-two sera were only positive in the Ag-ELISA and 72 only in the Ab-ELISA. The sensitivity and specificity of the Ag-ELISA and Ab-ELISA used for the diagnosis of pig cysticercosis in the Mayo-Danay division are presented in table 1. The validation criteria of the Bayesian model are shown in table 2.

Risk factor analysis

The factors that were considered as significant risks for pig cysticercosis are presented in table 3. The seroprevalence of cysticercosis in pigs was found to be associated with the pig owner's level of knowledge about the taeniasis–cysticercosis complex (odds ratio (OR) = 0.360, $P < 0.05$) and pig pen availability (OR = 0.45, $P < 0.05$).

Discussion

Risk factors for porcine cysticercosis

In 1980, the pig population in Cameroon was estimated at more than 1,000,000 and 60% of the pigs were located in the western part of the country. After an outbreak of African swine fiver (ASF) in 1982 (Nana-Nukechap & Gibbs, 1985), the pig population decreased in this part of the country. Since 1990, the north of Cameroon, free from ASF, emerged as the main provider of pork for the city of Yaoundé (Ndebi & Ongla, 2006). Pigs are kept in a traditional low-input breeding system (Njoya *et al.*, 1997), which favours infection with *T. solium* (Awa *et al.*, 1999). The results of this survey showed that 42.7% of households keeping pigs in the rural areas have no latrine facility and 76% of pig farmers use open-field defecation around the households. Even when a latrine was available this did not imply that it was used by the members of the household. In fact, these latrines were built to avoid problems with the sanitary authority, which controls the availability of latrines in the villages on a regular basis.

The coexistence of poor sanitary conditions and free roaming of domestic pigs (absence of pig pens; OR = 0.45; $P < 0.05$) certainly plays an important role in

Table 1. The true prevalence of porcine cysticercosis in Mayo-Danay and the sensitivity (Se) and specificity (Sp) of Ag-ELISA and Ab-ELISA estimated by means of Bayesian analysis (with 95% confidence intervals).

Combined test	True prevalence	Ag-ELISA		Ab-ELISA	
		Se	Sp	Se	Sp
Ag-ELISA/Ab-ELISA	0.266 (0.156–0.310)	0.895 (0.804–0.994)	0.947 (0.902–0.997)	0.585 (0.425–0.787)	0.754 (0.689–0.818)

Table 2. Model validation based on Bayesp, DIC and P_D used to estimate the prevalence of cysticercosis in Mayo-Danay division and the sensitivity and specificity of Ag-ELISA and Ab-ELISA.

Combined test	Bayesp	P_D_Pr	P_D_p	DIC_Pr	DIC_P
Ag-ELISA/Ab-ELISA	0.492	2.983	2.941	22.994	22.894

Bayesp, Bayesian-*p* values; DIC, Deviance Information Criterion; DIC_Pr, DIC values from posterior mean of the multinomial probabilities; DIC_P, DIC values from posterior mean of the parameter of the model using parent nodes.

the circulation of *T. solium* infection in the region. Free roaming of pigs is known to be an important risk factor for infection of pigs with *T. solium* (Sikasunge *et al.*, 2007). This is not the case in the west and north-west of Cameroon where direct defecation by people in pig pens is an important factor associated with transmission of cysticercosis (Zoli *et al.*, 1987; Shey-Njila *et al.*, 2003). In addition, pigs are privately slaughtered without meat inspection. When answering the questionnaire, pig keepers knew and recognized pig cysticercosis through tongue examination or in pig meat, but did not know the relationship between taeniasis in humans and the presence of cysticerci in pigs. This lack of knowledge about the transmission of *T. solium* (OR = 0.360; *P* < 0.05) may contribute to the maintenance of a high prevalence of pig cysticercosis in the region.

Prevalence of porcine cysticercosis

In an earlier epidemiological survey conducted in the Mayo-Danay division in 1999, 38.9% of the pigs were found to be seropositive using an ELISA for antigen detection (Assana *et al.*, 2001). The results of the ELISAs in the present study (24.6% seropositive by Ag-ELISA and 32.2% by Ab-ELISA) are somewhat lower but confirm that pigs are still highly exposed to *T. solium* cysticercosis. However, neither of the tests used for the diagnosis of cysticercosis is perfect (Dorny *et al.*, 2004, Krecek *et al.*, 2008). The Ab-ELISA, using a 14 kDa purified antigen (F3), is more specific but less sensitive than the Ag-ELISA (Assana *et al.*, 2007). It measures the exposure of the pigs to *T. solium*, but this does not imply that cysticerci are present. The Ag-ELISA, on the other hand, detects the presence of living cysts, but cross-reacts with *Taenia hydatigena*. The advantage of using both the Ab-ELISA and the Ag-ELISA and analysing the results in a Bayesian model is that the F3 antigen used in the former test does not react with serum samples from pigs infected with *T. hydatigena* (Assana *et al.*, 2007). However, the occurrence of *T. hydatigena* is not well known in northern Cameroon and should be studied further. The seroprevalence of cysticercosis using Ab-ELISA was higher than when using Ag-ELISA. This seems to indicate that a proportion of the pigs harbour only dead cysts or have specific transient antibodies against cysticercosis (Garcia *et al.*, 2001). In our study, 37% (42/114) of the serum samples positive in Ag-ELISA tested negative in Ab-ELISA. This discrepancy might be due to the lower sensitivity of the Ab-ELISA or to false-positive results in Ag-ELISA due to cross-reaction with *T. hydatigena*. Similar high proportions

Table 3. Univariate analysis of risk factors associated with seroprevalence of porcine cysticercosis in Mayo-Danay.

Variable	Level	Total number of pigs	Seropositive pigs (%)	Statistical significance	
				P	Odds ratio (95% CI)
Free-roaming pig system in dry season	No	30	5 (16.7)	0.298	1.691 (0.629−4.544)
	Yes	368	93 (25.3)		
Free-roaming pig in rainy season	No	344	90 (26.2)	0.077	0.491 (0.223−1.080)
	Yes	54	8 (14.8)		
Size of the household	<8	126	32 (25.4)	0.807	0.941 (0.578−1.533)
	≥8	272	66 (24.3)		
Duration of pig farming (years)	<5	77	22 (28.6)	0.371	0.775 (0.444−1.354)
	≥5	321	76 (23.7)		
Pig owner's level of knowledge of the taeniasis−cysticercosis complex	Poor	354	93 (26.3)	0.037	0.360 (0.130−0.940)
	Average and good	44	5 (11.4)		
Pig pen availability	No	49	16 (32.7)	0.016	0.446 (0.231−0.860)
	Yes	349	62 (17.8)		
Latrine availability	No	142	36 (25.4)	0.802	0.941 (0.586−1.512)
	Yes	256	62 (24.2)		
Latrine use	No	297	74 (24.9)	0.816	0.939 (0.554−1.593)
	Yes	101	24 (23.8)		
History of tapeworm infection in the household	No	341	84 (24.6)	0.991	0.996 (0.519−1.911)
	Yes	57	14 (24.6)		
Preference for cooking meat	Long cooking	68	17 (25.0)	0.937	0.976 (0.534−1.784)
	Grilled meat	330	81 (24.5)		

of positive sera in antigen detection found negative in antibody detection were obtained with Ab-ELISA using crude antigen from *T. solium* (Pouedet *et al.*, 2002), crude antigen from *T. crassiceps* (Dorny *et al.*, 2004) and also in a recent study using an enzyme-linked immunoelectro-transfer blot assay (EITB) based on affinity purified glycoproteins from *T. solium* cysticerci (Krecek *et al.*, 2008). In the absence of data from autopsy (slicing of all muscles), which is the gold standard, it is possible to use conditional dependence between two or more imperfect tests to estimate the true prevalence of a disease and its test parameters by using a Bayesian analysis (Dendukuri & Joseph, 2001; Berkvens *et al.*, 2006). In this study, we used conditional dependence between Ag-ELISA and Ab-ELISA to estimate the true prevalence of cysticercosis in the Mayo-Danay division. The Bayesian analysis indicated a true prevalence of 26.6%. This result is higher than the figure of 12% found by Pouedet *et al.* (2002) in western Cameroon, using a similar approach. The Bayesian analysis of these data revealed the sensitivity and specificity of the Ag-ELISA to be higher than those found with the Ab-ELISA (table 1). However, the diagnostic characteristics of the Ab-ELISA using F3 antigen and the EITB, the assay most commonly used for antibody detection in the diagnosis of cysticercosis (Tsang *et al.*, 1989), seem to be similar to those reported by Krecek *et al.* (2008), who used the same Bayesian approach.

In conclusion, this study clearly shows that porcine cysticercosis is hyperendemic in Mayo-Danay and that the pig-farming system contributes to maintaining this high level of infection. This situation calls for preventive measures to control pig cysticercosis in the region.

Acknowledgements

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