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Epidemiological survey of swine cysticercosis in two rural communities of West-Cameroon

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Abstract

To determine the prevalence of porcine cysticercosis, a survey was carried out in 27 villages belonging to two rural communities of West-Cameroon (Bafou and Bamendou). Between January and August, 2000, a total of 707 pigs were examined serologically and by tongue inspection. Serum samples were examined for circulating parasite antigen using a monoclonal antibody-based sandwich enzyme-linked-immunosorbent assay (Ag-ELISA) and for antibodies against cysticerci (Ab-ELISA). Seventy eight samples (11.0%) were found positive in the Ag-ELISA and 154 (21.8%) in the Ab-ELISA, while by tongue inspection on the same animals cysticerci were detected in 43 pigs (6.1%). Gibbs sampling using results of these three tests indicated that the estimated prevalence of porcine cysticercosis was 10.9%. Analysis of the Ag-ELISA results demonstrated that adult pigs showed a significantly higher seroprevalence (15%) than young ones (8.4%). There was no statistical difference in cysticercosis prevalence in pigs raised in households with or without a latrine. Animals that were reported to be usually confined were significantly less infected (9.9%) than free-roaming pigs (16.2%). Infection rates were significantly higher in pigs that had access to human faeces (13.8%) than those which did not have access (9.1%). This study has identified some community behavioural and environmental practices that should be modified to prevent continuous transmission of porcine cysticercosis.

Keywords: Pig–cestoda; *Taenia solium*; Cysticercosis; ELISA; Epidemiology; Cameroon

1. Introduction

Cysticercosis in man and swine is an infection caused by the larval stage of *Taenia solium*. In the life cycle of this parasite, humans are the definitive hosts because they harbour the adult

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tapeworm in the small intestine whereas pigs are the normal intermediate hosts. Humans can also serve as the intermediate host by accidental ingestion of *T. solium* eggs. Infection in pigs is facilitated by their coprophagic habits (Sarti et al., 1992).

In many developing countries, this disease constitutes a serious but sometimes under-recognised public health problem (Tsang and Wilson, 1995) and causes important economic losses because of condemnation of infested pork (Roberts et al., 1994). Poor sanitation and lack of veterinary control provide the conditions to sustain the life cycle of *T. solium* (Garcia et al., 1998).

In Latin America (Flisser, 2002), and some parts of Asia (Ito et al., 2002) and Africa (Geerts et al., 2002), cysticercosis has been reported as endemic. In Cameroon, few reports have been published about cysticercosis in pigs (Zoli et al., 1987; Nguekam, 1998; Awa et al., 1999). Recently, Assana et al. (in press) described a new focus of cysticercosis in the far-north province of the country. All these studies in Cameroon have been carried out in local slaughterhouses or in market places. However, little or no data exist on the epidemiology of cysticercosis of village pigs in smallholders' farms. We used three different diagnostic techniques in order to reliably estimate the real prevalence of porcine cysticercosis in two rural communities of West-Cameroon (Bafou and Bamendou). We also analysed the effects of the age of the pigs, presence or absence of latrines in the household, access to human faeces, system of pig rearing, and locality on the seroprevalence of porcine cysticercosis.

2. Materials and methods

2.1. Animals

Two hundred and ninety families, representing about 80% of the total number of pig raising households in two rural communities (Bafou and Bamendou, Menoua Division) in the western highlands of Cameroon were selected on the basis of accessibility. All pigs belonging to these households—except pregnant and nursing sows—i.e. 707 pigs (184 males and 523 females) were included in this study. The survey was performed between January and August, 2000, in 27 villages of these two communities (altitude: 1800 m above sea level; mean annual rainfall: 1872 mm; temperature: 16–25 °C).

2.2. Tongue inspection

The tongue test consisted of palpation and visual identification of nodules on the tongue. The pig was placed on its side, held by the neck and firmly restrained. A hard wooden rod was used to keep the mouth open. The tongue was pulled out, examined, and palpated throughout its base for the presence of cysticercosis nodules.

2.3. Serological tests

2.3.1. Serum samples

Blood samples were collected from 707 village pigs. The serum was separated and stored at −20 °C until tested. Each sample was tested in duplicate, and on each ELISA plate two

positive reference serum samples from local naturally infected pigs (*T. solium* cysticercosis confirmed at slaughter) and eight serum samples from *T. solium* cysticercosis-free pigs (negative at tongue palpation and originating from a local farm with good hygienic conditions and without any history of cysticercosis) were included.

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

The Ag-ELISA, which was initially developed for *T. saginata* cysticercosis (Brandt et al., 1992), was performed as described by Dorny et al. (2000) with slight modifications. The sera were pre-treated using trichloroacetic acid (TCA) and used in ELISA at a final dilution of 1/4. Two monoclonal antibodies (MoAb) were used in a sandwich ELISA. MoAb B158C11A10 was diluted at 5 μg/ml in carbonate buffer (0.06 M, pH 9.6) for coating and a biotinylated MoAb B60H8A4 (1.25 μg/ml in PBS–T20/NBCS) was included as detector antibody. The incubation was carried out at 37 °C on a shaker during 30 min for the coating of the first MoAb and during 15 min for all subsequent steps. The chromogen/substrate solution consisting of orthophenylene diamine (DAKO, #S2045) 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 (Labsystem Multiskan RC) at 492 nm.

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

Fresh cysticerci were collected from massively infected local pigs and were carefully dissected from host tissues. Then, they were washed repeatedly and stored at −20 °C. After thawing, the material was centrifuged at $3217 \times g$ for 30 min at 4 °C. The supernatant (cyst fluid) was collected and used as antigen in the ELISA. The optimal dilution of the antigen, serum and conjugate was determined by "checker-board titration".

The assay involved coating polystyrene ELISA plates (Nunc® Maxisorp) with 100 μl per well of cyst fluid antigen diluted at 1/2000 in carbonate buffer (0.06 M, pH 9.6), and incubating on a shaker during 30 min at 37 °C. The plates were washed once with PBS–Tween-20 (phosphate buffered saline + 0.05% Tween-20). Blocking was done by adding 150 μl per well of PBS–Tween-20 + 1% new born calf serum (PBS–T20/NBCS), and then the plates were incubated on a shaker during 15 min at 37 °C. Plates were emptied and 100 μl of test sera diluted at 1/300 in PBS–T20/NBCS was added (without washing) and incubated on a shaker at 37 °C for 15 min. After washing (three times), 100 μl of peroxidase conjugate (rabbit anti-pig IgG, SIGMA) diluted 1/30,000 in PBS–T20/NBCS was added and incubated on a shaker for 15 min at 37 °C. 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 *t*-test (Sokal and Rohlf, 1981).

2.4. Data collection and analysis

Information on the system of pig rearing, faecal access, and presence or absence of latrines was provided by the heads of the households and verified by direct observation. Pigs were classified as adult ($\geq$1 year) or young ($<$1 year).

For the analysis of the effects of pig age, presence or absence of latrines in the household, access to human faecal materials, system of pig rearing, and locality on the seroprevalence (Ag-ELISA) of porcine cysticercosis, the *z*-test for the equality of two proportions (Kanji, 1999) was used with a probability level of $P = 0.01$.

A Bayesian approach was used to draw inferences about cysticercosis prevalence and test properties (sensitivity, specificity) of the three tests (tongue inspection, Ag-ELISA and Ab-ELISA). A Gibbs sampling programme (Gamerman, 1997) was developed in Winbugs (available upon request to D. Berkvens). Gibbs sampling is a Monte Carlo Markov Chain technique, whereby the transition makes use of the full conditional distributions. This technique allows the simultaneous estimation of prevalence and test characteristics, combining the prior knowledge (previous surveys, expert opinion or simply a non-informative distribution) with the present survey results to obtain the posterior distribution for each of the parameters.

3. Results

Of the 707 pigs examined, 577 (81.6%) were usually kept in confinement and 130 (18.4%) were free roaming. Among the 290 pig-owning households visited (174 in Bafou and 116 in Bamendou), 33 (11.4%) did not have latrines, and in 154 (53.1%) of the households pigs had access to human faecal material.

Table 1 summarizes the data on the villages visited, and on the number of pigs found positive for cysticercosis by either tongue inspection, Ab-ELISA or Ag-ELISA. By tongue inspection, there was no statistical difference between the number of animals found positive in Bafou and Bamendou. The Ag-ELISA and Ab-ELISA, however, detected significantly higher number of positive cases in Bamendou than in Bafou. In four out of 27 villages, infected pigs could not be detected.

Nine animals were positive for cysticercosis by tongue inspection, but were negative in the Ag-ELISA. Twenty seven animals positive in the Ag-ELISA were negative in Ab-ELISA, while antibodies were absent in the serum of 17 pigs positive by tongue inspection (Table 2). Table 3 shows that twenty two animals were simultaneously declared positive in the three tests, while 521 were negative in all the tests used in this study.

The results of the Gibbs sampling applied to the data obtained from the three tests used (Ag-ELISA, Ab-ELISA and tongue inspection) are shown in Tables 4 and 5. The estimated prevalence with or without prior information about the sensitivity and specificity of the three tests is 10.9 and 12%, respectively.

Fig. 1 presents the percentage of positive samples (Ag-ELISA) in function of pig age, system of rearing, hygienic conditions of the household, presence or absence of latrines, and locality. There was no statistical difference in cysticercosis seroprevalence of pigs raised in households with or without latrines. Adult pigs showed a significantly higher seroprevalence

Table 1
Prevalence of porcine cysticercosis by tongue inspection, Ag-ELISA and Ab-ELISA in Bafou and Bamendou (West-Cameroon)

Localities	No. of samples collected	Positive at tongue inspection	Positive in Ag-ELISA	Positive in Ab-ELISA
Bafou community				
Bawouwoua	27	2	4	1
Lepia	28	0	2	0
Doumbouo Centre	7	0	0	0
Bassessa	8	0	0	0
Bafou Sp.	13	0	0	0
Fokamezo	7	0	1	0
Mezet	32	0	7	0
Tsingbeu	20	2	2	8
Tchoutsi	72	9	8	3
Fombet	29	3	3	14
Batsingla	27	1	3	11
Fonakeukeu	6	0	1	5
Bafou Chefferie	60	0	4	5
Lepouo	25	0	0	12
Melouong	39	5	1	2
Sub-total	400	22 (5.5%)	36 (9%)	61 (15.3%)
Bamendou community				
Bamendou Lumière	4	0	0	3
Mbouo	37	7	7	1
Tchueffi	33	4	0	3
Mentsa	30	2	4	2
Bamendou Chefferie	21	0	2	13
Dedeng	28	0	3	2
Bamendou Sp.	19	0	0	0
Balefock	25	2	6	9
Nkotsa	21	1	2	12
Batoula	41	1	7	24
Metchou	24	4	10	16
Léo	24	0	1	8
Sub-total	307	21 (6.8%)	42 (13.7%)	93 (30.7%)
Total	707	43 (6.1%)	78 (11.0%)	154 (21.8%)

Table 2
Comparison of three different tests two by two used for the detection of cysticercosis in village pigs ($n = 707$)

	Tongue inspection		Ab-ELISA	
	+	−	+	−
Ag-ELISA				
+	34	44	51	27
−	9	620	103	526
Ab-ELISA				
+	26	128		
−	17	536		

Table 3
Correlation between the results obtained by three different tests for the detection of cysticercosis in village pigs ($n = 707$)

No. of samples	Tests		
	Tongue inspection	Ag-ELISA	Ab-ELISA
521	−	−	−
99	−	−	+
29	−	+	+
15	−	+	−
5	+	−	−
4	+	−	+
12	+	+	−
22	+	+	+

Table 4
Results of the Gibbs sampling without any prior information about the sensitivity and the specificity of the three tests (Ag-ELISA, Ab-ELISA and tongue inspection)[a]

Node	Mean	Standard deviation
P	0.120	0.017
Se1	0.465	0.064
Se2	0.858	0.071
Se3	0.683	0.060
Sp1	0.992	0.005
Sp2	0.989	0.008
Sp3	0.844	0.015

[a] Test 1: tongue inspection; test 2: Ag-ELISA; test 3: Ab-ELISA; P: prevalence; Se: sensitivity and Sp: specificity.

Table 5
Results of the Gibbs sampling with prior information on the sensitivity and the specificity of the tongue inspection (Se: 70% and Sp: 98%, slightly modified according to Gonzalez et al. (1990)), the Ab-ELISA (Se: 67.6% and Sp: 98.2%, Nunes et al. (2000)) and the Ag-ELISA (Se: 86% and Sp: 99%, slightly modified according to Nguekam (1998))[a]

Node	Mean	Standard deviation
P	0.109	0.015
Se1	0.618	0.041
Se2	0.872	0.061
Se3	0.716	0.061
Sp1	0.992	0.005
Sp2	0.981	0.009
Sp3	0.860	0.014

[a] Test 1: tongue inspection; test 2: Ag-ELISA; test 3: Ab-ELISA; P: prevalence; Se: sensitivity and Sp: specificity.

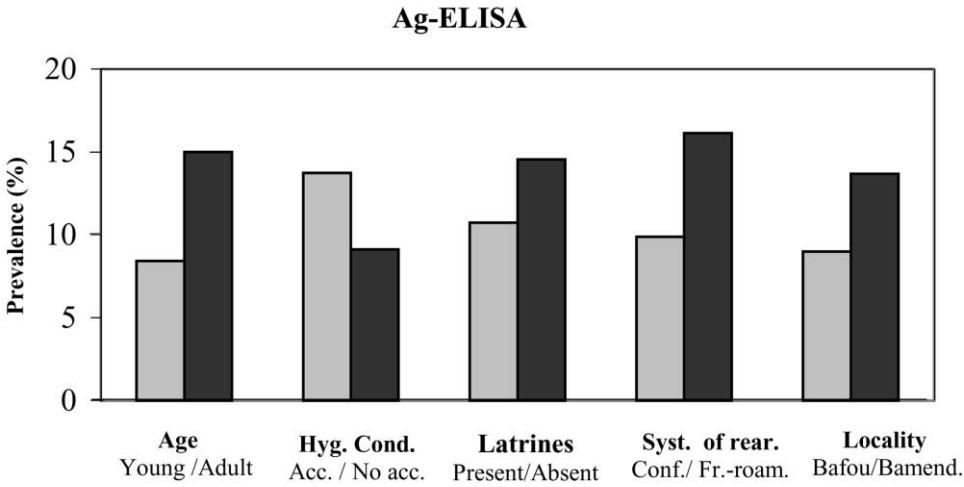

Fig. 1. Prevalence of cysticercosis (Ag-ELISA) in function of pig age, rearing system, household hygienic conditions, presence or absence of latrines, and locality (Hyg. Cond.: hygienic condition; Acc.: access to human faeces; No acc.: no access to human faeces; Syst. of rear.: system of rearing; Conf.: confinement; Fr.-roam.: free roaming; Bamend.: Bamendou.).

(15%) than young ones (8.4%). Pigs that were reported by their owners to be usually confined were significantly less infected (9.9%) than those that free-roamed (16.2%). Animals which had access to human faeces were significantly more infected (13.8%) than those which did not (9.1%).

4. Discussion

The prevalence of porcine cysticercosis using tongue inspection was 6.1%. This result is distinctly higher than the 2.3% rate reported by Nguekam (1998) in the bordering Divisions of Bamboutos and Mifi (West-Cameroon). In contrast, it is lower than the 24.6% reported by Zoli et al. (1987) in the Menoua Division.

However, the prevalence of porcine cysticercosis estimated by Ab-ELISA (21.8%) and Ag-ELISA (11.0%) was almost trice or double, respectively, than by tongue inspection. In this study, we used an antigen detection ELISA which has proved to be highly specific (99.1%) and sensitive (84.6%) for pigs infected with cysticercosis (Nguekam, 1998). The cut-off level for the Ag-ELISA was calculated on the basis of the average OD of serum samples of eight pigs which originated from a farm without history of cysticercosis and which were negative at tongue palpation. Although these pigs have not been autopsied, the OD of their sera was very low (<0.02), so that it can be assumed that they were free of cysticercosis. However, the use of a larger number of sera from uninfected control pigs, which are representative for the local pig population, would undoubtedly increase the reliability of the Ag-ELISA. Since the detection limit of this test is unknown, it is probable

that the 9 animals found positive by tongue inspection but negative by Ag-ELISA harboured a number of cysts below the detection limit of this technique. Another explanation might be that the nodules discovered on the tongue of those animals were not caused by cysticerci but by other lesions (e.g. scars after mechanic injuries). These arguments might also be valid for the 17 positive cases by tongue inspection but negative in the Ab-ELISA. With the Ab-ELISA, antibodies were detected in 154 (21.8%) sera representing the number of animals in both communities, which had a history of contact with *T. solium* oncospheres. According to Nunes et al. (2000) the Ab-ELISA using cyst fluid of *T. solium* cysticerci as antigen had a sensitivity of 67.6% and a specificity of 98.2%. The fact that 27 animals, which were positive in the Ag-ELISA and thus probably harbouring living cysts, were not identified in the Ab-ELISA is very surprising. This confirms the low sensitivity of the antibody detection ELISA. However, it might also indicate that some false positive reactions occurred in the Ag-ELISA.

The Gibbs sampling programme was used assuming that the three tests were statistically independent conditional on the true disease status of the subject. With or without prior information on the sensitivity and specificity of the three tests used, the programme gave an estimated prevalence of cysticercosis of 10.9 and 12.0%, respectively. The advantage of this approach is that it allows a much more reliable estimate of the disease prevalence than a survey based on the results of a single test. It is striking that the obtained figures were very close to the prevalence figures recorded in the Ag-ELISA (11.0%). These results indicate the endemicity of the disease in both rural communities (Bafou and Bamendou) and complement previous reports based on data collected at slaughterhouses and market places (Zoli et al., 1987; Awa et al., 1999; Nguekam, 1998). The latter results, however, have to be interpreted with caution due to the high mobility of animals between markets of different localities within the country.

The seroprevalence (Ag-ELISA) of porcine cysticercosis in Bamendou (13.7%) was significantly ($P < 0.01$) higher than in Bafou (9%) and at least 15.3% of pigs examined in Bafou and 30.3% in Bamendou were carrier of antibodies against cysticerci. The higher figures in Bamendou are probably due to the high percentage of people who used the pigpen as a toilet. In this area, human defecation along the roads and in crop fields was also common. Total seroprevalence by Ab-ELISA (21.8%) was lower than the result (38%) reported by Zoli et al. (1987) in a survey in the same Menoua Division. This decrease of the seroprevalence (Ab-ELISA) indicates that the general hygienic conditions in this area have been improved by the time although insufficiently.

For the epidemiological analysis, the data of the Ag-ELISA were used because these were very close to the estimated prevalence using Gibbs sampling. The results of the present study demonstrated that adult pigs showed a significantly ($P < 0.01$) higher seroprevalence (15%) than young ones (8.4%). One obvious reason is that old animals have been more exposed to the infection than young ones. These results are in agreement with those reported by Sarti et al. (1992) in Mexico. In contrast, in some studies no relationship between age and prevalence of infection with cysticerci of *T. solium* was recorded (Sakai et al., 1998; Nguekam, 1998). There was a significant difference ($P < 0.01$) in cysticercosis prevalence between pigs that were usually confined to pens (9.9%) and those which were free roaming (16.2%). These figures are similar to those obtained by inquiries about the hygienic conditions of the households, which showed animals with access to human faeces to be more infected (13.8%)

than those without access (9.1%). Although most households had latrines, they were often not constructed in a manner that excluded access of pigs. A similar situation was reported by Schantz et al. (1998) in Mexico, Guatemala and Peru. The high percentage (53.1%) of households visited where pigs had access to human faecal material was remarkable.

5. Conclusion

The results of this study conducted in two rural communities (Bafou and Bamendou) demonstrated that porcine cysticercosis still remains an important problem in West-Cameroon. Bamendou appeared to be the most infected community. Multiple factors, including pig husbandry practices, household sanitation, and hygiene were shown to be associated with parasite transmission. In order to reduce the infection risk, it is necessary to intensify meat inspection, improve sanitary infrastructure, and educate the population. Recent surveys showed that many inhabitants of both communities either ignore or are ignorant of the danger to which they expose themselves by eating meat with cysticerci and by using pigpens as a toilet. Most of them did not understand the association between the presence of cysticerci in the animal and the tapeworm infection in man (Djou, Personal communication).

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