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Short communication

# Old focus of cysticercosis in a senegalese village revisited after half a century

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ABSTRACT

The objective of this epidemiological study was to determine whether cysticercosis and especially neurocysticercosis is endemic in Soutou village about half a century after the 1962 outbreak. This study was carried out from September 2009 to February 2010. It involved a questionnaire administration, serology, treatment, coproscopy and neuro-imaging. Four hundred and three serum samples were collected from the village people, which covered 94% of the village population. By using a parallel combination of the antigen-detection ELISA and the enzyme-linked immunoelectrotransfer blot (EITB) a cysticercosis seroprevalence of 11.9% (95% CI: 8.9–15.4%) was found. Cerebral CT-scans showed that 23.3% (10/43) of the seropositives were affected by neurocysticercosis. Four out of these 43 (9.3%) were tapeworm carriers. Seropositivity was significantly associated to older age groups (41–60 years old; $p = 0.001$ and 61–91 years old; $p = 0.028$) and absence of a household toilet ($p = 0.001$). It can be concluded that Soutou village is an active focus of *Taenia solium* cysticercosis about 50 years after the first reported epidemic outbreak.



## 1. Introduction

There is a lack of data about the prevalence of human and porcine cysticercosis in West Africa. Geerts et al. (2004) reviewed the situation and found that large-scale surveys for human cysticercosis were only carried out in Togo (Houinato et al., 1998) and Benin (Dumas et al., 1989) with a seroprevalence of 2.4 and 1.3%, respectively. Based on the currently available information the prevalence of *Taenia solium* cysticercosis both in man and pigs in West Africa is generally much lower than in Eastern and Southern Africa (Geerts et al., 2004). For Senegal there are only a few reports available. In 1962, an outbreak of human cysticercosis was reported in a village called Soutou, which is located in the Bignona 'département' of the Casamance region of Senegal. In this village cysticercosis was affecting 23 out of 529 people (Collomb et al., 1964). Extra two cases of neurocysticercosis were reported in the same area in 1975 (Dumas et al., 1976). Recently, a seroprevalence of porcine cysticercosis of 26.7% (8/30) using an antigen detection ELISA (Ag-ELISA) was found in Soutou in 2008 (Secka et al., 2010). This high proportion of infected pigs tends to indicate that the environment is contaminated with *T. solium* eggs. This study was undertaken to examine the current situation of cysticercosis and especially neurocysticercosis in Soutou about half a century after the 1962 outbreak.

## 2. Materials and methods

### 2.1. Study area

Soutou is a small village situated in the 'Arrondissement de Tenghory', 'Communauté rurale de Tenghory', Bignona 'département' in the region of Ziguinchor in southern Senegal (Casamance). It is located at latitude 12° 52.488 north and longitude 016° 15.459 west. It is about 15 km from the administrative town of Bignona. The majority of the village population belongs to the Jolla ethnic group and are faithful Christians. The health centre of the village, run by the resident Catholic Mission, became operational since 1965. Most of the villagers are farmers growing crops and rearing animals. Pigs are the predominant animal species reared in this village. They are kept indoors only during the cropping season and then left under free range system for seven months of the year.

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### 2.2. Sample and data collection

The study population consisted of people present in Soutou village during the six days sampling period in September 2009. All individuals present in the village during this period, except 24 children less than four years of age were investigated for cysticercosis. Following the signing or thumb printing of a consent form, about four millilitres of blood were collected from the brachial vein of 403 people covering 94% of 427 inhabitants present. The serum samples were kept at −20° C until tested. At the same time, people were searched for the presence of subcutaneous nodules on their arms, faces and legs. Each sampled person was also interviewed using a standard questionnaire. The questionnaire was divided into four sections (personal identification, epilepsy history, epilepsy predisposing factors, and cysticercosis predisposing factors). A villager was considered to suffer from epilepsy when he or she reported to have had 2 or more epileptic seizures with an interval of more than 24 h and not post-partum or caused by fever, cranial trauma or metabolic disorder (ILAE, 1989).

### 2.3. Diagnostic tests

All 403 collected sera samples were subjected to both antigen detection enzyme-linked immunosorbent assay (Ag-ELISA) (Brandt et al., 1992; Dorny et al., 2004) and enzyme-linked immunoelectrotransfer blot (EITB) (Tsang et al., 1989) to detect circulating antigens and antibodies against *T. solium*, respectively. Affinity purified glycoproteins from *T. solium* cysticerci (in-house prepared by one of the authors, FG) were used as antigen in the EITB.

The cut-off for the Ag-ELISA was calculated as the average optical density (OD) of 8 negative samples plus three standard deviations, assuring a test specificity of 99.9% (Sokol and Rohlf, 1981). The 8 negative serum samples were obtained from healthy persons without any history of cysticercosis in Gambia. A serum sample was considered as positive when the ratio (mean OD of duplicate test sample/OD cut-off) was $\geq$1.2. A serum sample (dilution 1:50) was considered positive in EITB if at least one of the seven diagnostic glycoprotein antigens of *T. solium* was detected.

Forty three out of 48 seropositive study subjects, positive at Ag-ELISA and/or EITB, underwent contrast computed tomography (CT-scan) at Ziguinchor regional hospital to diagnose neurocysticercosis. The radiological criterion for diagnosing neurocysticercosis was based on the observation of viable and/or calcified cysticerci in the brain sections of the study subjects. The CT-scanning and interpretation of images were done by one of the co-authors (L.B., radiologist), who was informed about the serology results. Faecal samples from 43 niclosamide (Taeniadex®) treated seropositive people (16 females aged 7–84 years and 27 males aged 10–72 years) were examined for taeniid eggs using Ritchies formalin ether concentration method (Ritchie, 1948) and direct faecal examination under microscope. The faecal samples were collected from the first defecation after treatment within 24 h. The dosage rate of niclosamide varied from 2 g for adults to 0.5 g for children weighing less than 10 kg. One expelled worm, from a 29-year old niclosamide treated lady, was subjected to PCR-RFLP (Rodriguez-Hidalgo et al., 2002) to determine its species.

### 2.4. Data collection and analysis

Data were collected from all study participants through a direct face-to-face interview using questionnaires. The questionnaire was administered by the first author (A.S.) using a combination of English, French, and local languages Wollof and Jola. Seropositivity was defined as being positive in either Ag-ELISA or EITB or both tests.

The significance of explanatory variables such as the study subject's sex, age category, marital status, religion, household toilet, washing hands and management of pigs, in relation to seropositivity at either test was first evaluated using a stepwise backward selection of estimators process ($p < 0.1$). The retained explanatory variables were subsequently tested in a robust multivariate logistic regression model.

### 2.5. Ethical clearance

After obtaining the ethical approval from the National Ethics Committee for health research of the Senegalese Ministry of Health, further clearances were pursued from the medical authorities down the chain of command up to village level prior to the start of the study. The villagers were then sensitized on the objectives of the study in collaboration with the village medical person. Oral consent was allowed by the Ethics Committee because many villagers were illiterate. All study subjects signed or thumb printed (together with a signature of a literate witness) the consent form before we drew blood samples and administered the questionnaires.

## 3. Results

### 3.1. Characteristics of the study population

The majority of the 403 participants belong to the Jola ethnic group, profess the Christian faith and are born in Soutou village. Forty six percent are under 21 years old, 24% between 21 and 40 and the remaining 30% between 41 and 91. Five persons (one male and 4 female) reported to have experienced epileptic seizures. The age-at-onset of the seizures was 10, 12, 18, 50 and 60 years. Two of them were taking medication. Functional toilets were reported to be available in the households of 396 (98%) respondents, whilst 3 (1%) used neighbours toilet, and 4 (1%) defecated in the bush. The real use of the toilets by the villagers was confirmed by the resident medical personnel of the village. Village toilets are basically covered round pits, with a small opening where users squat, and an enclosure wall. The faeces decompose and the pit naturally closes up with soil when it gets full. All respondents except for 6% claimed to wash their hands with soap after using the toilet. The main source of drinking water at the village level is water drawn from dug wells. All respondents, except for 8 (2%), claimed to have pigs at their households which are seasonally confined. Pork is consumed by all respondents in cooked or grilled form. Fifteen (4%) respondents reported to have expelled taeniid worms during their early ages. Subcutaneous nodules were not observed in any of the villagers. Seropositivity was found to be significantly associated with older age (41–60 years old – $p = 0.001$ and 61–91 years old – $p = 0.028$) and absence of household toilet ($p = 0.001$).

### 3.2. Cysticercosis–taeniosis complex

The results of the different tests are given in Table 1. The seroprevalence of cysticercosis reached 7.7% (95% CI: 5.5–10.7%) in each of the serological tests. A parallel combination of test results (positive in at least one test) gave a prevalence of 11.9% (95% CI: 8.9–15.4%) whilst the serial (positive at both tests) produced a prevalence of 3.5% (95% CI: 1.9–5.8%).

The results of the four combinations of the two serological tests are summarised in Table 2.

Five of the 48 seropositive individuals failed to undergo a cerebral CT-scan for various reasons. Cyst structures compatible with cysticerci were found using CT-scan in 10 of the 43 seropositive people. The estimated number of cysts varied from 2 to 39 (7 out of 10 had less than 5 cysts) and all were localised in the brain parenchyma. In 6 people only calcified cysts were found whereas

**Table 1**
The results of the various tests to detect (neuro)cysticercosis and taeniosis in the population of Soutou.

| Diseases and tests applied | Sample size | Number positive | Percentage positive (95% C.I.) |
|---|---|---|---|
| *Cysticercosis* | | | |
| Ag-ELISA (test 1) | 403 | 31 | 7.7% (5.3–10.7) |
| EITB (test 2) | 403 | 31 | 7.7% (5.3–10.7) |
| Test 1 or/and Test 2 | 403 | 48 | 11.9% (8.9–15.4) |
| *Neurocysticercosis* | | | |
| Contrast computed tomography (CT-scan) | 43 | 10 | 23.3% (11.8–38.6) |
| *Taeniosis* | | | |
| Direct examination | 43 | 2 | 4.7% (0.6–5.8) |
| Ritchie formol ether method | 43 | 4 | 9.3% (2.6–22.4) |
| Worm expulsion | 43 | 1 | 2.3% (0.1–12.3) |

one person harboured only viable cysts and 3 had both viable and calcified cysticerci. One of the 10 CT-scan positives reported to have epileptic seizures. Incidentally, he also got the strongest reaction at both serological tests. Intraventricular dilatation was found in three of the seropositives. All the cysticercosis infected persons who were eligible for a treatment got the opportunity to undergo a free appropriate treatment (albendazole alone or combined with carbamazepine in case of epilepsy).

*Taenia* spp. eggs were found in 4 out of 43 niclosamide treated villagers by the Ritchie method two of which were also positive at direct examination. From one of the 4 egg positives a tapeworm could be collected which was identified by PCR-RFLP as *T. saginata.*

## 4. Discussion

This is the first follow-up study of human cysticercosis in Soutou since the last epidemic in the early 1960s and mid 1970s. As seropositivity was defined as being positive in at least one test, the seroprevalence was 11.9%. Seropositivity was found to be significantly associated with older age which is in agreement with the observations of other authors (Sarti et al., 1992; Sarti et al., 1994) in some rural villages of Michoacan state and Morelos, Mexico and in West Cameroon (Nguekam et al., 2003). The association of seropositivity with absence of household toilets confirms that cysticercosis is associated with a low sanitary level. Three out of 4 villagers defecating in the bush and two out of three using neighbours' toilet were seropositive. Seventy one percent (5/7) of these people did not wash their hands with soap after defecating hence are more prone to faecal-oral contamination that could lead to cysticercosis.

The seroprevalence detected by Ag-ELISA alone (7.7%) which is also equal to that detected by EITB is higher than the 3% reported in the West province of Cameroon (Nguekam et al., 2003), 1.3% in Benin (Houinato et al., 1998), and 2.4% in Togo (Dumas et al., 1989), which are all cysticercosis endemic regions or countries. It is similar to the 7% reported by Vanderick and Mbonyingabo (1972) based on autopsy findings in Rwanda in 1972 which was a hyperendemic country at that time. Although autopsy findings are lacking for Soutou, the available evidence indicates that Soutou is a hyperendemic focus of *T. solium.* Hyperendemic cysticercosis was also reported by Garcia-Noval et al. (1996) in 2 rural Guatemalan villages (seroprevalence of 10 and 17%) based on a similar approach as this study by using questionnaires, serology and CT-scan.

**Table 2**
Number of observations recorded for the four combinations of the two serological test results.

| Ag-ELISA | EITB | Number of observations |
|---|---|---|
| – | – | 355 |
| + | – | 17 |
| – | + | 17 |
| + | + | 14 |
| Total | | 403 |

The overall seropositivity of 11.9% emanates from the increased probability for detecting both active and inactive cysticercosis because of the use of both antigen and antibody tests in parallel. The Ag-ELISA is reported to have a sensitivity of 94.4% and a specificity of 97.6% (Erhart et al., 2002; Prado-Jean et al., 2007) whilst the EITB has a sensitivity of 98% and a specificity of 100% according to Tsang et al. (1989). However, since serological tests are not the gold standard for detecting cysticercosis, this overall seropositivity might be overestimated by the detection of transient antibodies (Garcia et al., 2001) or underestimated because of the presence of false negatives. Some subcutaneous cysticerci might have been missed since only the face, arms and exposed legs were examined. This might explain why no cases of subcutaneous cysticercosis were observed even though the subjects were asked if they had any nodules on their bodies.

There were three CT-scanned study subjects that had only intraventricular dilatation which could have been caused by a blockage from tumours, cysts or other infectious agents. Based on the fact that they were positive at either or both Ag-ELISA and EITB, we suspect they had neurocysticercosis even though no cysts were seen.

## 5. Conclusion

Although no *T. solium* tapeworm could be identified the results of this study together with the fact that 26.7% of the village pigs are positive in the Ag-ELISA (Secka et al., 2010) indicate that the village of Soutou is an active focus of *T. solium* cysticercosis. In 1962, Collomb et al. (1964) reported that 23 out of 529 people (4.3%) in the same village were affected by cysticercosis (based mainly on clinical examination; X-ray and electro-encephalogram were used in a few cases). These authors ascertained a case of cysticercosis by the presence of subcutaneous nodules (plus biopsy and histological examination in some of them) and/or the presence of calcified cysts in the brain or the muscles by radiological examination. Today using serological techniques, this figure has increased to 11.9% whereas neurocysticercosis was present in at least 10 out of 403 (2.5%) based on a limited number of CT-scans. Although we cannot directly compare the figures of both studies, it is obvious that *T. solium* cysticercosis remains a serious problem in this village. Over the last 50 years the sanitary situation of the people and the use of toilets have significantly improved, but apparently there have been no major changes in the way pigs are kept in this village. This indicates that free roaming pigs plays a major role in the maintenance of the life cycle of *T. solium*. Even when the majority of the population uses toilets as is the situation in Soutou, it is apparent that a few people defecating in the bush is enough to keep the cycle going.

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