



Neurocysticercosis in a rural population with extensive pig production in Angónia district, Tete Province, Mozambique

Yunus Amade Assane^{a,b}, Chiara Trevisan^{c,*}, Clara Maria Schutte^a,
Emilia Virginia Noormahomed^{b,d}, Maria Vang Johansen^c, Pascal Magnussen^{c,e}

^a Department of Neurology, University of Pretoria, Pretoria, South Africa

^b Medical Faculty Eduardo Mondlane University, Maputo, Mozambique

^c Section for Parasitology and Aquatic Diseases, Department of Veterinary Disease Biology, Faculty of Health and Medical Sciences, University of Copenhagen, Denmark

^d Department of Medicine, University of California, San Diego, CA, United States

^e Centre for Medical Parasitology, Department of International Health, Immunology and Microbiology, Faculty of Health and Medical Sciences, University of Copenhagen, Denmark

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ABSTRACT

Neurocysticercosis (NCC) is an important neurological disease in countries with high prevalence of *Taenia solium* infection and is emerging as a serious public health and economic problem. The aim of this study was to estimate the prevalence of NCC in Angónia district, Tete province, Mozambique based on: prevalence of human *T. solium* cysticercosis assessed by antigen Enzyme-linked Immunosorbent Assay (Ag-ELISA) seropositivity, history of epilepsy, and brain computed tomography (CT) scan results. A cross sectional study was conducted between September and November 2007 in Angónia district. Questionnaires and blood samples were collected from 1,723 study subjects. Brain CT-scans were carried out on 151 study subjects with confirmed history of epilepsy. A total of 77 (51.0% (95% CI, 42.7–59.2)) and 38 (25.2% (95% CI, 18.5–32.9)) subjects met the criteria for definitive and probable diagnosis of NCC, respectively. *T. solium* Ag-ELISA seropositivity was found in 15.5% (95% CI, 12.8–16.2) of the study subjects. The estimated life time prevalence of epilepsy was 8.8% (95% CI, 7.5–10.2). Highly suggestive lesions of NCC were found on CT-scanning in 77 (71.9%, (95% CI, 62.4–80.2)) of the seropositive and 8 (18.1%, (95% CI, 8.2–32.7)) of the seronegative study subjects, respectively. The present findings revealed a high prevalence of NCC among people with epilepsy in Angónia district. Determination of effective strategies for prevention and control of *T. solium* cysticercosis are necessary to reduce the burden of NCC among the affected populations.

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1. Introduction

Neurocysticercosis (NCC) is an important neurological disease in countries with high prevalence of *Taenia solium* infection and is reported to be a re-emerging problem in high income countries (Fabiani and Bruschi, 2013; Serpa and White, 2012). The condition develops when a person ingests parasite eggs present in stool of patients with taeniosis (Roman et al., 2000). Taeniosis is acquired by eating raw or undercooked infected pork. While patients with taeniosis generally exhibit little or no clinical signs and symptoms,

a different scenario is observed in patients with NCC. Epilepsy is a common clinical presentation (Carabin et al., 2011; Del Brutto and Del Brutto, 2012; Del Brutto and Garcia, 2013; Winkler et al., 2009b) and leading cause of morbidity in patients with NCC (Dewhurst et al., 2013; Tegueu et al., 2013). Epilepsy is considered a major health problem in low and middle income countries, where the prevalence has shown to be much higher than in high income countries (Ngugi et al., 2010). Two systematic reviews have recently provided an updated estimate for the overall prevalence of epilepsy in sub-Saharan Africa but did not include data from Mozambique (Ngugi et al., 2010; Paul et al., 2012). Ndimubanzi et al. (2010) estimated in a systematic review that NCC occurred in 29% of people with epilepsy, while Quet et al. (2010) revealed a significant association between cysticercosis and epilepsy in a meta-analysis including only African studies (Ndimubanzi et al., 2010; Quet et al., 2010). Several studies on porcine cysticercosis have been carried

* Corresponding author at: Department of Veterinary Disease Biology, Faculty of Health and Medical Sciences, University of Copenhagen, Dyrlægevej 100, 1870 Frederiksberg C, Denmark.

E-mail address: Chiara@sund.ku.dk (C. Trevisan).

out in Tete province, Mozambique and have provided an indication that the zoonotic parasite is wide spread in the area (Pondja et al., 2010; Pondja et al., 2015). The burden it poses on the human population has not been assessed so far. The aim of this study was to estimate the prevalence of NCC in Angónia district, a rural area of Mozambique based on: prevalence of human *T. solium* cysticercosis assessed by antigen Enzyme-Linked Immunosorbent Assay (ELISA) seropositivity, history of epilepsy, and brain computed tomography (CT) scan results.

2. Materials and methods

2.1. Ethical statement

The study protocol was reviewed and approved by the Ethical Committee of the Medical Faculty of Eduardo Mondlane University, the National Bioethics Committee of Mozambique, the Danish National Committee on Biomedical Research Ethics, Denmark and the Faculty of Health Sciences Ethics Committee at the University of Pretoria, South Africa.

Written informed consent was obtained from all study subjects before interview and sample collection. Study subjects who could not sign their name used fingerprint. Participation was voluntary and study subjects were free to withdraw from the study at any time. All patients' data from the study were anonymized. Treatment was offered free of charge according to national treatment guidelines. If at any time the study subject developed any clinical complication or side effect, the study subject, parent or guardian was instructed to report immediately to the local health care center.

2.2. Study area and population

The study was conducted in Angónia district, Tete province, Mozambique. The district has two administrative posts – Ulongue and Domue – and sixteen towns which are subdivided into 307 villages and communities. In 2005 the total population of the district was 330,378 people. The main economic activity of the district was mixed farming, including pig rearing. Health care access was limited to one rural hospital, four health centres and three health points. Inhabitants of the area also consult traditional healers (Direcção Distrital de Agricultura de Angónia, 2005).

2.3. Study design and sampling

A community-based cross sectional study was conducted between September and November 2007. Sample size estimation was calculated based on an expected prevalence of human cysticercosis of 20% in the study area (Vilhena and Bouza, 1994). Using single proportion calculation and the following formula $n = Z^2 p(1-p)/d^2$, the sample size was estimated to be 1600 in the district ($p = 0.2$, $SE = 0.0196$) (Wayne, 1999). Sampling with probability proportional to size was used to select the villages. Systematic random sampling was used to select the households to be included. At each household a list of all members was obtained from which one of the available participants was then randomly selected (Chromy, 2008). Criteria of eligibility were: living in the household. Prior to the start of the survey, an initial field visit was made in order to explain the purpose of the study to the local authorities and villagers. In total 1,723 study subjects originating from 18 villages and towns of Angónia district were included.

2.4. Questionnaire

A field tested questionnaire, based on the one developed by the Cysticercosis Working Group of Eastern and Southern Africa, was administered to the study subjects to record data on risk

factors for *T. solium* cysticercosis and other related information, such as pig ownership, pork consumption and history of epileptic seizures (Mwanjali et al., 2013). All questionnaires were translated from English to the local language Chichewa, and back translated to English. Interviews were conducted by paramedical staff that spoke the local language and who had received training during the preparation phase of the study. Study subjects, who during the questionnaire reported having had epileptic seizures in the past, were further interviewed by the principal investigator (first author) to confirm a history of epilepsy.

2.5. Definition of epilepsy

Epilepsy was defined, according to the International League Against Epilepsy (ILAE), as two or more unprovoked epileptic seizures separated by at least 24 h and unrelated to acute metabolic disorders or to withdrawal of drugs or alcohol (Fisher et al., 2005; ILAE International League Against Epilepsy, 1993). Lifetime prevalence of epilepsy (LPE) was defined as the proportion of patients identified with a history of epilepsy at any time, regardless of treatment or recent seizure activity. Lifetime prevalence of epilepsy included patients with active epilepsy or epilepsy in remission with or without treatment (ILAE International League Against Epilepsy, 1993).

2.6. Blood sampling

A venous blood sample (4.5 ml) was obtained from each study subject. Serum was separated by centrifugation in the field and stored at -20°C in cryotubes (Nunc, Roskilde, Denmark) in the blood bank of the Rural Hospital. The samples were transported from Tete to Maputo in dry ice and stored at the division of Parasitology, Medical Faculty, Eduardo Mondlane University, in Mozambique before transport to School of Veterinary Medicine, University of Zambia, in Zambia. Here the serum samples were tested for circulating antigens of the metacestode of *T. solium* (Ag-ELISA (B185/B60) using the Ag-ELISA assay (Brandt et al., 1992; Dorny et al., 2000; Nguekam et al., 2003). The Ag-ELISA assay has shown to have a sensitivity of 90% and a specificity of 98% in detecting current infection with *T. solium* metacestodes (Praet et al., 2010). Negative reference control sera from local people and one positive control reference serum from a patient with confirmed cysticercosis were included in each ELISA run. The optical density (OD) of each serum sample was compared with the mean of the eight negative reference sera at a probability level of $p\text{-value} = 0.001$ to determine the result using a modified Student's *t*-test (Sokal and Rohlf, 1995). The ELISA ratio was calculated by dividing the OD of the sample by the calculated cut-off value of the eight negative controls. An ELISA ratio bigger than one (ratio > 1) was considered positive (Dorny et al., 2004; Somers et al., 2006).

2.7. Brain CT-scan

Study subjects with confirmed history of epilepsy were transported to Beira Central Hospital, in Sofala province to have a CT-scan of the brain to ascertain presence of lesions suggestive of NCC. Brain CT-scan examinations were carried out using a Somatom emotion helicoidal, version A45A (Germany). In case lesions suggestive of NCC were found, the intravascular contrast agent Ultravist-370 was used. Each patient was monitored by a physician before, during and after contrast injection. Brain CT-scans were analysed independently by two neurologists from Beira and Maputo Central Hospital.

Brain lesions were classified as: vesicular, colloidal, nodular-granular and calcified (Escobar and Weidenheim, 2002). Any cystic

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