



Research paper

Severe seizures in pigs naturally infected with *Taenia solium* in TanzaniaChiara Trevisan^{a,*}, Ernatus M Mkupasi^b, Helena A Ngowi^b, Björn Forkman^c, Maria V Johansen^a^a Department of Veterinary Disease Biology, University of Copenhagen, Dyrlægevej 100, 1870 Frederiksberg C, Denmark^b Department of Veterinary Medicine and Public Health, Sokoine University of Agriculture, P.O. Box 3021, Morogoro, Tanzania^c Department of Large Animal Sciences, University of Copenhagen, Grønnegårdsvej 8, 1870 Frederiksberg C, Denmark

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ABSTRACT

Neurocysticercosis (NCC) caused by *Taenia solium* is a serious neurological disease. In humans neurological symptoms have been thoroughly studied and documented, however, there is limited information on clinical signs in pigs infected with *T. solium* cysticerci. Among the scientific community, it is in fact believed that pigs with NCC rarely show neurological signs. The aim of this study was to describe clinical manifestations associated with NCC in pigs and correlate the manifestations to the number and distribution of cysticerci in brains of naturally infected pigs in Tanzania. Sixteen infected and 15 non-infected control pigs were observed for 14 days during daylight hours, and subsequently videotaped for another 14 consecutive days using close circuit television cameras. All occurrences of abnormal behaviour (trembling, twitching, mouth and ear paralysis, ataxia, dribbling, salivating, eye blinking, walking in circles) were recorded. At the end of the recording period, pigs were slaughtered and their brains dissected, cysticerci counted and locations noted. During the recording period, two infected pigs were observed having seizures. Some of the observed autonomic signs during a seizure were chewing motions with foamy salivation and ear stiffening. Motor signs included tonic muscle contractions followed by a sudden diminution in all muscle function leading to collapse of the animal. Stereotypic walking in circles was observed on several occasions. At dissection, both pigs had a high number of brain cysticerci (241 and 247 cysticerci). The two pigs with seizures were also older (36 months) compared to the others (18.3 months, ± 8.2 standard deviation). Results of this study have shown that pigs with NCC can develop clinical signs and suffer from seizures like humans with symptomatic NCC. Results of this study could potentially open up a new experimental pathway to explore the aetiology of neurological symptoms in humans with NCC associated epilepsy.

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1. Introduction

Neurocysticercosis (NCC) caused by the larval stage of the pig tapeworm *Taenia solium* is a serious neurological disease (White, 1997). In humans, epilepsy, headache and impaired vision are common clinical presentations of NCC and leading causes of morbidity. Dementia, learning difficulties and changes in cognition are often secondary sequelae of humans with NCC (Garcia et al., 2003). In endemic areas NCC is estimated to be responsible for one third of all late onset epilepsy cases (Ndimubanzi et al., 2010) and causes

substantial health and economic burdens in the affected populations (Carabin et al., 2006; Praet et al., 2009; Torgerson and Macpherson, 2011; Trevisan et al., 2016).

Whilst in humans these neuropathologic and clinical aspects have been well studied and documented, there is limited information on the symptomatology the parasite causes in pigs. A number of researchers have previously reported the rarity of signs developed in association with porcine cysticercosis and a limited number of studies exploring the latter (Garcia et al., 2003; Saenz et al., 2008). However, in a study conducted in India, eye blinking, tearing and excessive salivation were reported as clinical signs suggestive of porcine NCC. To confirm presence of cysticerci in the brain and correlate the signs to NCC, the pigs underwent magnetic resonance imaging (MRI) examination (Prasad et al., 2006). Since the animals included in the study of Prasad et al. (2006) were chosen based

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on the signs observed by animal caretakers and as the pigs had to be sacrificed for gross pathology and histopathology examination at the end of the MRI scans, the signs could not be reconfirmed. Furthermore, as the study design did not include control pigs, it is not known if the reported signs occurred due to the health conditions of the animals or their environment during the study. Another study by [Mkupasi et al. \(2014\)](#) reported clinical manifestations such as dullness, sluggishness, somnolence, apathy and loss of consciousness in pigs naturally infected with *T. solium* cysticerci. The observed clinical manifestations were only considered as suggestive of NCC because no control pigs were available in that study.

In pigs, seizures have been observed to be caused by salt poisoning, dehydration or pseudo rabies ([Gelberg, 2010](#)), however, the zoonotic parasite *T. solium* has never been reported as an agent causing seizures in this species.

The aim of the present study was to describe possible clinical manifestations associated with NCC and correlate the manifestations to the number, location and distribution of cysticerci in brains of naturally infected pigs in Tanzania.

2. Materials and methods

2.1. Animals

The study was carried out at the experimental animal facilities at Sokoine University of Agriculture (SUA), Morogoro, Tanzania. Infected pigs were purchased from farmers in the rural area of Kongwa district, Dodoma region, an area where *T. solium* is known to be highly prevalent ([Mkupasi et al., 2013](#)). To ascertain presence of infection, the pigs were diagnosed by tongue examination ([Dorny et al., 2004](#)). Pigs with more than three cysticerci under the tongue were included in the study. As the sensitivity of the diagnostic method is low, non-infected pigs were purchased from smallholder farmers in villages of Morogoro rural district, Morogoro region, where the prevalence of porcine cysticercosis is known to be low (Ngowi, personal communication).

Pigs not sexually mature (maturity determined by age (younger than 6 months) and size (lower than 50 cm in height) or in poor condition (under two thirds of the average weight of 40 kg for a healthy adult pig and visibly ill (covered with ectoparasites and/or with injuries)) were excluded. Infected pigs were purchased first and non-infected pigs were matched with infected ones a posteriori by applying the same exclusion criteria. On arrival of the pigs at SUA, all animals were checked for hard ticks and lice. To eliminate possible confounders (endoparasites and ectoparasites) pigs were treated twice, at an interval of two weeks, with a subcutaneous injection of 0.3 mg/kg of ivermectin (ivermectin 1.0%) ([Barragry, 1987](#)). Ivermectin was considered safe, as no adverse effects were observed in a study on ivermectin in pigs with cysticercosis ([Mkupasi et al., 2013](#)).

2.2. Study design

An observational study was carried out on 16 pigs naturally infected with *T. solium* and 15 non-infected pigs. A parallel group design was formed. As the disease cannot spread between pigs, three to four infected pigs and their respective controls were randomly chosen and housed together. All pigs were housed under equal conditions in pens (4 × 3 m) with a cement floor and walls. The pens were cleaned every day and the pigs were fed twice a day with commercial dry pig feed. Water was provided *ad libitum* and after morning feeding the animals were provided with forage (*Leucaena leucocephala* or *Amaranthus spinosus*). The mean room temperature of the stable was of 25 °C. Light was present from 6:00 am until 7:00 pm.

Pigs were kept at the experimental animal facilities for one month. During the first two weeks of acclimatisation, the pigs were observed during the day (7:30 am until 6:00 pm). Abnormal occurrences such as: trembling, twitching, uncontrolled movements of rostrum, mouth rigidity and ear paralysis, eye blinking, dribbling, salivating, body stiffening, ataxia, tonic/clonic contractions, panting, collapse of the animal and stereotypic walking in circles were recorded when observed.

After two weeks of acclimatisation, the pigs were continuously videotaped for 14 consecutive days using close circuit television cameras (Velleman® CCTVPROM16). One camera was mounted above each pen in a central position, permitting a top down view of the whole pen. Each pig was colour-marked using coloured stock markers on its back and on its sides to allow individual identification on the video recordings. The videos of the animals where abnormal occurrences were observed during the acclimatisation period were scanned for abnormal behaviour using fast forward video visualization.

At the end of the video recording period, all 31 pigs (infected and non-infected) were slaughtered by a local butcher. After bleeding, the carcasses of the pigs were transported to the pathology laboratory of the Department of Veterinary Pathology, Faculty of Veterinary Medicine at SUA. There the animals were decapitated at the atlanto-occipital joint ([Agerholm, 2011](#)). To remove the brain from the cranium the procedure described by [Agerholm \(2011\)](#) was adopted.

To evaluate the distribution of the cysticerci, the cerebral part of the brain was divided into its left and right hemisphere. Thereafter each hemisphere was divided into frontal, temporal, parietal and occipital lobe and cerebellum using sulci as landmarks. Each lobe was carefully sliced to examine and enumerate cysticerci. The cysticerci were evaluated to determine whether they were located in the extra-parenchymal part (parts not completely surrounded by brain tissue) i.e. subarachnoid dorsal and subarachnoid base or in the parenchymal part of the frontal, temporal, parietal and occipital lobe and cerebellum. Cysticerci that could not be located were counted as cysticerci found on the cutting board. Furthermore, the cysticercus developmental stage (vesicular, colloidal or calcified) was recorded ([Fleury et al., 2004](#)).

2.3. Seizure classification

Seizures were classified according to a modified version of the International League Against Epilepsy (ILAE) seizure system developed for dogs ([Licht et al., 2002](#)). The seizure classification summary (Table S1) used is available in Supplementary material. Seizures were divided into four categories: Partial, complex partial, partial with secondary generalization and generalized ([Licht et al., 2002](#)).

2.4. Data analysis

Data were entered in Microsoft Excel 2010 and analysed using the statistical software R (R Core Team, 2014). Descriptive statistics were performed to compute means and proportions of cysticerci in different parts of the brain. Multiple logistic regression was adopted to assess the effect of age, total number and location of cysticerci on presence of seizures. P-values lower than 0.05 were considered statistically significant.

2.5. Research ethics

Practices employed in the study were approved by SUA, Morogoro, Tanzania (Ref. no. RPGS/R/AS/42/2014) and in accordance with the national guidelines of ethics for health research and to

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