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ORIGINAL RESEARCH

Sarcocystis infection in slaughtered cattle in Zango abattoir, Zaria, Nigeria

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Abstract

Background*: Sarcocystis* infection is a parasitic zoonosis, which may cause acute and fatal clinical diseases in susceptible cattle. When raw or undercooked infected beef is consumed by man, it could result in intestinal sarcocystosis.

Aim: This study aimed at determining the prevalence of *Sarcocystis* infection in slaughtered cattle in Zaria, Nigeria.

Materials and Methods: A cross sectional study was conducted in which oesophagus and diaphragm samples were collected from 200 slaughtered cattle and analysed by pepsin-hydrochloric acid digestion and stained with Giemsa. Histological sections of tissues were prepared and stained with haematoxylin and eosin.

Results: Eighty-five (42.5 %) were positive for *Sarcocystis* species. Sarcocysts ranged from 228.8 to 1215 µm in length and 46.93 to 114.40 µm in width. Sarcocysts were all microscopic in nature and 99.0 % had thin cyst wall (< 1 µm), while 4 % had thick cyst wall (3.61 to 7.22 µm). *Sarcocystis cruzi* and *S. hominis* were the identified species. Age, sex and breed were not determinants of the infection ($p > 0.05$). Seventy-five (88.2 %) and 56 (65.9 %) cattle had sarcocysts in the oesophagus and diaphragm respectively. There was a significant difference in the distribution of sarcocysts between the oesophagus and diaphragm ($p < 0.05$).

Conclusion: This study has established in the study area the prevalence of *Sarcocystis* infection in cattle using tissue digestion method and histology. The identified species were of veterinary and public health importance.

Keywords: cattle, histology, Nigeria, *Sarcocystis*, tissue digestion

Introduction

Sarcocystosis is a parasitic zoonosis caused by species of *Sarcocystis*, an intracellular protozoan parasite in the Phylum Apicomplexa and Family Sarcocystidae. The infection is characterized by cyst formation in muscular tissues (muscular sarcocyctosis) in the intermediate host or colonisation of the lamina propria of the intestines (intestinal sarcocystosis) in the definitive host [1]. *Sarcocystis* has a requisite two-host life cycle based on a prey-predator (intermediate-definitive) host relationship [2]. *Sarcocystis*, once regarded as a non-pathogenic parasite, has been found to be associated with disease conditions in both animals and man [3, 4]. There are three species of *Sarcocystis* affecting cattle; *Sarcocystis cruzi*, *S. hominis* and *S. hirsuta*. *Sarcocystis cruzi* is the most common [1] and pathogenic species affecting cattle leading to abortion, reduced milk yield, neurologic signs, loss of weight, hair loss and death (fatal cases), depending on the species and number of sporocysts ingested [1]. Abortion and placentitis has been reported in pregnant cows naturally infected with *Sarcocystis* [5]. Humans acquire the infection by eating infected raw or undercooked beef containing mature sarcocysts. The current status of *Sarcocystis* infection in cattle in Nigeria is unknown. Available information dates back to the work of Kudi in 1989 [6], hence this study. The objective of this study was to determine the prevalence of *Sarcocystis* infection and to identify the species of *Sarcocystis* affecting slaughtered cattle in Zaria, Nigeria by using pepsin-hydrochloric acid digestion method and histology.

Materials and Methods

Study area: The study area covered Zaria town of Kaduna state, Nigeria. Zaria is one of the major cities in Kaduna state and as well a local government area, in North-west geo-political zone of Nigeria. Zaria lies in the northern guinea savanna zone of Nigeria with coordinates; 11.0667° N, 7.7000° E [7]. The occupation of Zaria inhabitants is primarily agriculture [7]. Staples are guinea corn and millet; cash crops include cotton, groundnut and tobacco. The major abattoir is located in Zango but there are also slaughter slabs in Sabon gari and Agoro.

Study design: Cross-sectional study.

Table-1. Age specific prevalence of *Sarcocystis* species in tissues (oesophagus and diaphragm) of slaughtered cattle in Zango abbatoir, Zaria, Nigeria

Age group	Total sampled	No. positive	Age specific rate (%)	P value
5- 8½ years	131	60	45.8	0.193
>8½ years	69	25	36.2	
Total	**200**	**85**	**42.5**	

(χ^2 = 1.694, df = 1) α = 0.05

Table-2. Sex specific prevalence of *Sarcocystis* species in tissues (oesophagus and diaphragm) of slaughtered cattle in Zango abbatoir, Zaria, Nigeria

Sex	Total sampled	No. positive	Sex specific rate (%)	P value
Male	65	31	47.7	0.303
Female	135	54	40.0	
Total	**200**	**85**	**42.5**	

(χ^2 = 1.062, df = 1) α = 0.05

Table-3. Breed specific prevalence of *Sarcocystis* species in tissues (oesophagus and diaphragm) of slaughtered cattle in Zango abbatoir, Zaria, Nigeria

Age group	Total sampled	No. positive	Breed specific rate (%)	P value
White Fulani	180	78	43.3	0.474
Sokoto Gudali	20	7	35.0	
Total	**200**	**85**	**42.5**	

(χ^2 = 0.512, df = 1) α = 0.05

Table-4. Distribution of sarcocysts in diaphragm and oesophagus of slaughtered cattle in Zango abattoir Zaria, Nigeria

Tissue	N	Mean Rank	P value
Diaphragm	200	191.00	0.043
Oesophagus	200	210.00	

*Mann-Whitney U = 18100.00

Sample collection: Between January 2011 and July 2011, 200 slaughtered cattle which included 65 males and 135 females of 5 to 12 years old in Zango abattoir of Zaria, Nigeria were examined for the presence of sarcocysts and bradyzoites in muscular (oesophagus and diaphragm) tissues. The tissues were first examined grossly and 25 g of oesophagus and diaphragm were collected from each animal and transferred to the Parasitic Zoonoses laboratory of the Faculty of Veterinary Medicine, Ahmadu Bello University Zaria, for microscopic examination.

Sarcocystis infection was classified into positive and negative. The cattle were classified into 2 age groups (5-8½ years and greater than 8½ years) based on visual inspection of the teeth, and into 2 breeds (White Fulani and Sokoto Gudali). White Fulani or Bunaji cattle is a white, black-eared and medium-horned breed, and is the most numerous and widespread of all Nigerian cattle breeds. The Nigerian National Livestock Resource Survey (NNLRS) estimated that they represent some 37% of the national herd [8]. They are found from Lagos to Sokoto, Katsina and Kano States and spread across the North central geo-political zone. [8]. There are two types of Gudali in Nigeria – the Sokoto Gudali (or Bokolooji) and the Adamawa Gudali. The Sokoto Gudali is a uniform cream, light grey or dun, the dewlap and skin folds are highly developed and the horns almost absent. The NNLRS estimated that they represent some 32% of the national herd [8].

Digestion method: The method of Dubey *et al* (1989) [1] and its modification [9] were used for digestion of the muscles. Briefly, 25 g of each sample was minced, placed into a stomacher bag containing 5 ml of normal saline and homogenized using a stomacher. The homogenate was suspended in 20 ml of digestion medium (1.3 g pf pepsin (BDH), 3.5 mL of 1% HCl and 2.5 g NaCl in 500 mL of distilled water) and digested for 1 hour at 37°C in a water bath. After digestion, the mixture was centrifuged at 1500 g for 5 minutes and the sediment stained with Giemsa and examined by optical microscope at x 400 magnification for detecting bradyzoites. An animal was said to be positive for *Sarcocystis* infection, if either the oesophagus or diaphragm contained sarcocysts/bradyzoites in the digested tissue.

Positive samples fixed in formal saline were submitted for histologic sectioning stained with haematoxylin and eosin (H&E). Photomicrographs of typical sarcocyst or bradyzoites were taken using a digital microscope camera (Tucsen®). The length, width and cyst wall thickness of the sarcocysts were taken using a calibrated micrometer eyepiece for the differentiation of *Sarcocystis* species and compared with reference values [1,10].

Statistical analysis: Data collated at the end of the study were analyzed using Statistical Package for Social Science (SPSS) version 17.0 (SPSS Inc. Chicago, IL, USA) and subjected to statistical analysis using descriptive statistics employing frequencies and percentages. Chi-square test of association was used to establish association between the infection status and variables such as age, sex and breed of the animal. Mann-Whitney test was used to compare the distribution of sarcocysts between oesophagus and diaphragm. P values less than 0.05 were considered significant.

Results

The overall prevalence rate of *Sarcocystis* infection in the 200 examined cattle, based on detection of bradyzoites (Figure I) by tissue digestion was 42.5 %. All sarcocysts observed were microscopic in nature. They ranged from 228.8 to 1215.5 µm in length and 46.93 to 114.40 µm in width. Of the positive samples, 99.0 % were *S. cruzi*, having a thin cyst wall (< 1 µm) and 4.0 % *S. hominis* (Figure 2), having a thick cyst wall (3.61 to 7.22 µm). There was no significant association (p > 0.05) between *Sarcocystis* infection and age, sex and breed of cattle (Tables 1, 2 & 3).

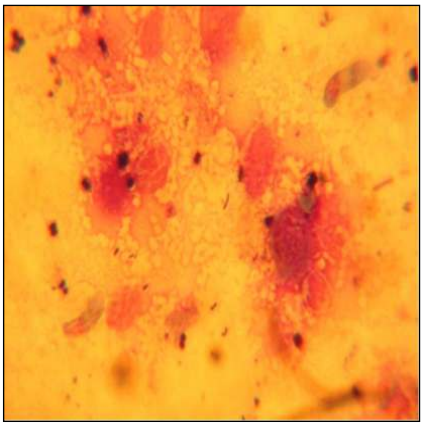

Figure-1. Photomicrograph of Giemsa stained bradyzoites in the oesophagus of cattle (optical microscope x 400 magnification using Tucsen® microscope camera)

Figure-2. Photomicrograph of *Sarcocystis* in the diaphragm of cattle stained with H&E (optical microscope x 400 magnification using Tucsen® microscope camera)

Seventy-five (88.2%) and 56 (65.9%) cattle had sarcocysts in the oesophagus and diaphragm respectively. There was a significant difference in the distribution of sarcocysts between the oesophagus and diaphragm ($p < 0.05$) (Table-4).

Discussion

Cattle become infected with *Sarcocystis* when oocysts/sporocysts of the parasite are ingested while grazing. The high prevalence (42.5 %) of *Sarcocystis* observed in cattle in this study is in contrast to 0 % prevalence rate reported by Kudi [6]. The increased detection rate of the parasite may be due to the modification in the technique of the earlier work. The method was modified by mincing and homogenizing the tissues before digestion and staining of tissue sediments with Giemsa stain. These procedures probably enhanced detection of bradyzoites in tissue smears. In addition, the source of animals may contribute to the high prevalence recorded. Presently in Nigeria, most cattle are purchased from neighbouring countries in contrast to the early 1970s and 1980s when the country could rely on her own cattle population. Perhaps, the animals acquired the infection from these sources before transportation into the country. Fayer [2] reported that sarcocysts can persist for months or years in the tissues of intermediate hosts.

Also, cattle farm owners/nomadic cattle rearers in Nigeria use dogs as security tools (dog shepherds) to protect their animals from danger. For nomads, they graze their cattle over various pastures moving from one location to another. The presence of dogs and other definitive hosts including non-human primates in the grazing pastures of the animals ensures shedding of the infective oocysts into the environment, which in turn infect the animals. Moreso, Sporocysts or oocysts of *Sarcocystis* remain viable for many months in the environment, and they may be further spread or protected by invertebrates [1].

The *Sarcocystis* species observed based on the morphometric features of the sarcocysts were *S. cruzi* and *S. hominis*. *Sarcocystis cruzi* was identified based on its microscopic nature, thin cyst wall and sarcocysts that were less than 500 µm in length. Some of the sarcocysts were up to 1215.5 µm in length with thick cyst wall and were microscopic in nature and identified as *S. hominis*. In histologic sections, *S. hominis* is difficult to distinguish from *S. hirsuta*. However, sarcocysts of *S. hominis* is microscopic in nature whereas *S. hirsuta is macroscopic* at meat inspection [11]. In this study, no macroscopic cyst was observed. The cyst walls of *S. hominis* and *S. hirsuta* can be differentiated ultrastructurally [1]. The finding of *S. hominis* in cattle in this study poses a public health risk because *S. hominis* is pathogenic in humans [1, 12].

Sarcocystis cruzi is the most prevalent and pathogenic species in cattle where it causes abortion, weight loss, neurologic sign, fever and death [1]. The result obtained in this study is similar to Savini *et al*., [13] who recorded a 52 % prevalence rate in Western Australia. Da Silva [14] reported 100 % prevalence rate in Rio Grande do Sul State, Hamidinejat *et al*., [9] reported 100 % prevalence rate in Ahvaz Khouzestan, south west of Iran while More *et al*., [15] reported 71.5% of *S. cruzi* cysts from beef cattle in Argentina. The result of this study supports earlier reports about *S. cruzi* being the most prevalent species worldwide.

In the present study, age, sex and breed were not significantly associated with *Sarcocystis* infection in cattle which disagrees with the finding of Savini *et al.*, [13] who reported that *Sarcocystis* infection in cattle in Western Australia was influenced by age, sex, environmental and management factors. In that study, cattle included in the study were less than 1½ years to greater than 4 years of age. *Sarcocystis* infection significantly dropped in cattle greater than 4 years of age. In the present study, the age range of cattle sampled was from 5 to 12 years (older cattle). This factor could have masked the effect of age on *Sarcocystis* infection, as calves were not sampled. However, a lower prevalence was observed in the older cattle, when compared to those between 5 to 8½ years, although the difference was not significant. Host immune response to the infection may be responsible for the fall in prevalence in the older cattle.

Sex was also not a determinant of the infection in

cattle, which is in contrast to the findings of Savini *et al*., [13] who reported a significantly higher prevalence of *Sarcocystis* infection in males than in females. In the present study, a higher prevalence was found in males than in females, although the difference was not significant. Breed was also found not to influence *Sarcocystis* infection in cattle, which may indicate that the two breeds of cattle studied had equal chance of being infected by *Sarcocystis* species.

Previous studies have shown that sarcocysts were most commonly seen in the oesophagus than any other parts of the body in cattle [13, 16]. The result of this study agrees with these findings. This is indicative that oesophagus is a good sampling site for sarcocysts in cattle for future investigations. Similar finding was observed in goats [17].

Conclusion

This study has established the prevalence of *Sarcocystis* infection in slaughtered cattle in the study area. The identified species were of veterinary, public health and economic importance, and age, sex and breed were not determinants of the infection.

Authors' contribution

All authors contributed equally. All authors read and approved the final manuscript.

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Competing interests

Authors declare that they have no competing interest.

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