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Histopathological Survey of Sarcocystosis in Slaugthered Animal Tissues in Benue State, Nigeria

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Abstract

Sarcocystosis is an important and common disease of domestic animals, yet not much is known about it in Benue State. This research was conducted during the wet season, from April to September, 2021 with the aim to survey the rate of contamination of slaughtered ruminants and pigs with Sarcocystis species using histopathological method. The study comprised of 1200 carcasses examined macroscopically, subsequently, tissue samples from skeletal muscle, heart, esophagus, tongue and diaphragm were considered for pathologic studies using the Hematoxylin and Eosin staining technique. The results showed that the highest rate of infection were in skeletal muscles of pigs in Makurdi (33.6 %). Infection was associated with location of both cattle and pigs ($P < 0.05$) but no significant difference ($P>0.05$) with sex and age of the animals. Macrocysts of white color, oval shape and size range of approximately 2-5 mm were observed in 12 skeletal muscles of the pigs, 5 of which had both microcysts and macrocysts. Sarcocyst shapes of two distinct types, elongated/fusiform and oval of varying intensities were also observed. To the best of our knowledge, these results represent the first demonstration of this parasites in animals of Benue State. The results indicate that Sarcocystis species infection is widely distributed in animals slaughtered for meat. The close proximity between livestock and humans on farms, and the frequent poor sanitary conditions in human dwellings strongly suggest an effective life cycle of the parasite. Further studies on molecular analysis is required for clear identification of different species in order to provide better strategies for zoonotic infection control in Nigeria.

Keywords: *Sarcocystis*, Ruminants, Pigs, Benue

Introduction

The parasite *Sarcocystis* is common in a wide variety of vertebrates and is one of the most widely spread protozoa in nature. In order for the lifecycle of the majority of *Sarcocystis* species to be completed, both a definitive (carnivore) and an intermediate (herbivore) host must be present (Dubey *et al*., 1989). The parasite causes sarcocystosis in both animals and man, some of which are zoonotic (Fayer *et al*., 2015). Cattle (*Sarcocystis cruzi*), goats (*Sarcocytsis capracanis*) and pigs (*Sarcocytis suihominis*) are intermediate hosts for some *Sarcocystis* species with felids and canids acing as definitive hosts (Chhabra and Samantaray 2013). Infection of farm animals is sometimes associated with the reduction in quality and quantity of meat, wool and fiber, resulting in important economic losses (Rubiola *et al*., 2020). This study was performed with the aim of estimating the prevalence of sarcocystosis in animals slaughtered in some abattoirs in Benue State, Northern Nigeria, during the wet season.

Materials and Methods

Study area

Benue State is geographically located on latitude 7.74° North and longitude 8.51° East and has 104m elevation above sea level. The mean monthly rainfall ranges from 150 mm to 180 mm, and the mean monthly temperature ranges from 27 °C to 38 °C. The state has the Benue River running through it. Inhabitants are mostly civil servants and farmers who consume and domesticate goats, pigs and cattle. Tissue samples were collected from three (3) abattoirs, namely: Makurdi, Otukpo and Adikpo, North-Central Nigeria, during the wet season period between the months of April and September, 2021 from animals (cattle, goats and pigs) slaughtered for human consumption.

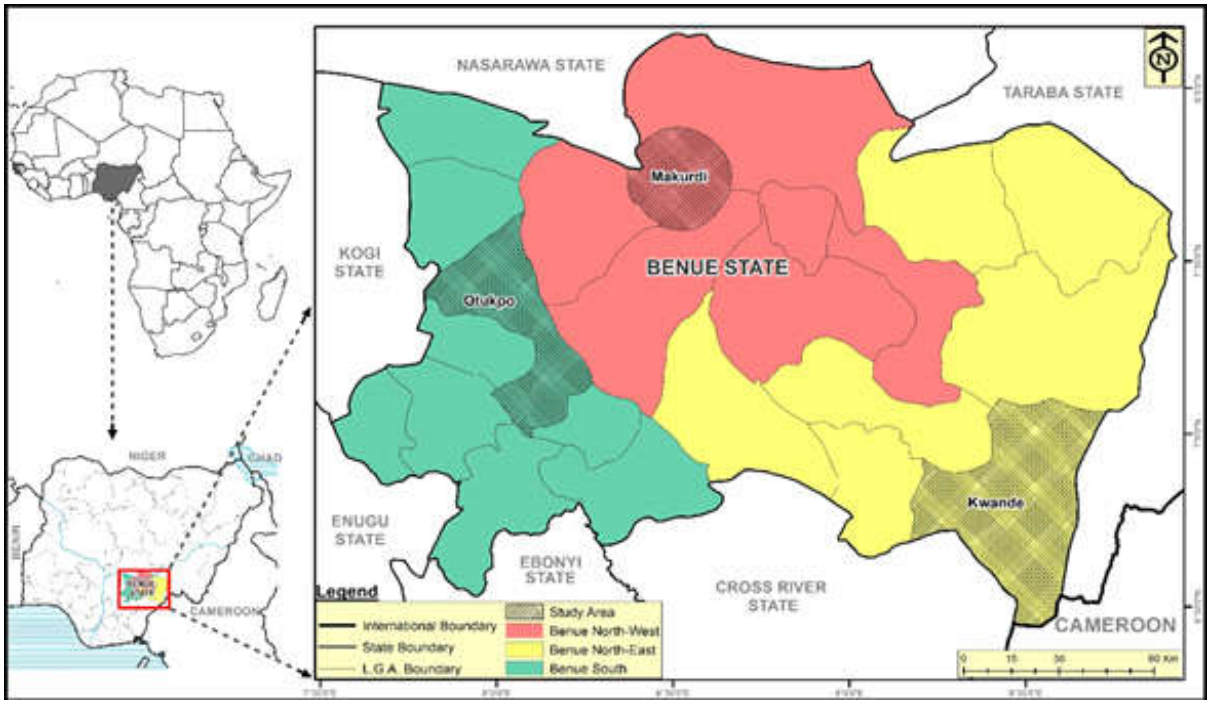


Figure 1: Sampling Location (Abattoir) in Study Area (Benue State)

Sampling

About 5g each of tissues from tongue, esophagus, heart, diaphragm and skeletal muscle were randomly collected from 1200 animals (goats, cattle and pigs), comprising young and adult animals of both sexes. The sexes of the animals were determined by physical examination of slaughtered animals while the age was ascertained by eruption of permanent incisors teeth (Chhabra and Samantaray 2013). The animals were categorized into two groups according to their age, those less than 2 years old were

considered young while those 2years and above were considered adult (Al-Saadi *et al*., 2020). Samples were kept in separate bags labelled with codes connoting the animal type, tissue and location. The samples were placed in box containing ice packs and transported to the laboratory. All procedures were conducted in accordance with the moral code for the use of animals according to the University of Agriculture, Makurdi, animal ethics regulations.

Macroscopic Examination

Animal tissues (tongue, esophagus, heart, diaphragm and skeletal muscle) were examined macroscopically, for pathological lesions and *Sarcocystis* macrocysts. Handheld macroscope was also used to magnify these animal tissues for detailed view (Farhad, 2014; Al-Saadi *et al*., 2020).

Histological Detection of cysts

Tissue sections (5µ) were obtained with a microtome and stained with Haematoxylin and Eosin (H&E) (Gill, 2009). Sarcocysts were identified according to the morphological keys by Dubey *et al*. (2016) and were counted per tissue section (area under 22 mm^2). Photomicrographs were taken using a digital microscopic eyepiece (Scope Photo 3.0; Version 3.0 of 2003-2007).

Statistical Analysis

Data was analyzed using SPSS version 21.0. The overall prevalence of infection was presented in percentages; Chi square was done to compare obtained data. The values of $P<0.05$ were regarded as statistically significant.

Results

Macroscopic Examination

The gross tissues examined appeared apparently normal, with no detectable colorations or pathological lesions. However, varying number (5- 8) of macrocysts of varying sizes from 2-5 µm were detected in 12 skeletal muscle samples and 5 others which had both macrocysts and microcysts, showing heavy infestation in some cases. This is as shown in plates 1 and 2.

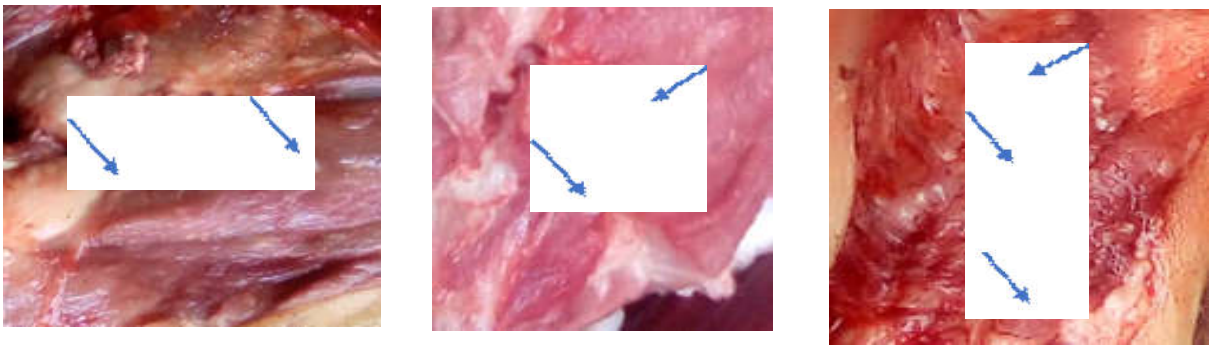


Plate 1: Infestation of skeletal muscle of Adult Male Pig in Makurdi with macrocysts (blue arrows)

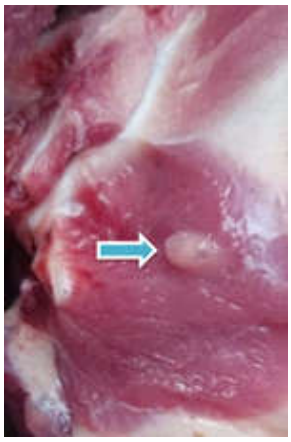


Plate 2a: Isolated macrocyst of 3mm

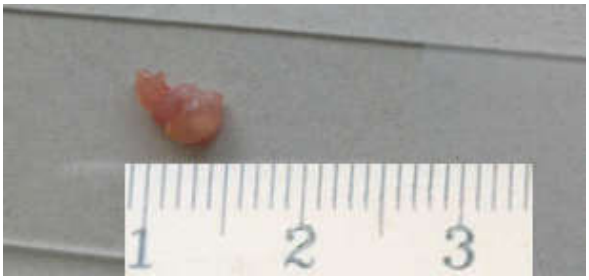


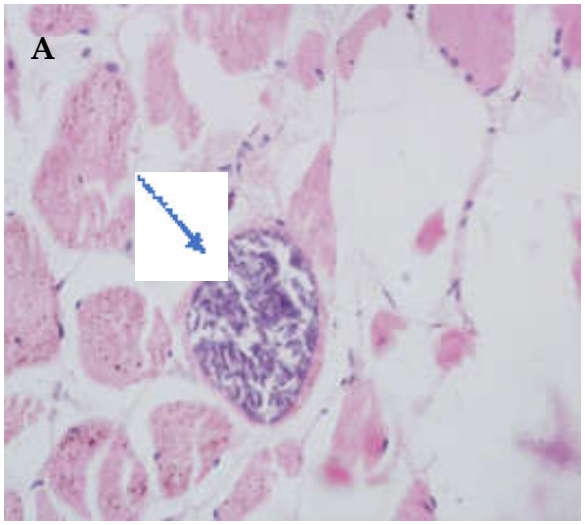
Plate 2b: Isolated macrocyst scale bar in mm from plate 2a

Histopathological/Microscopic Findings

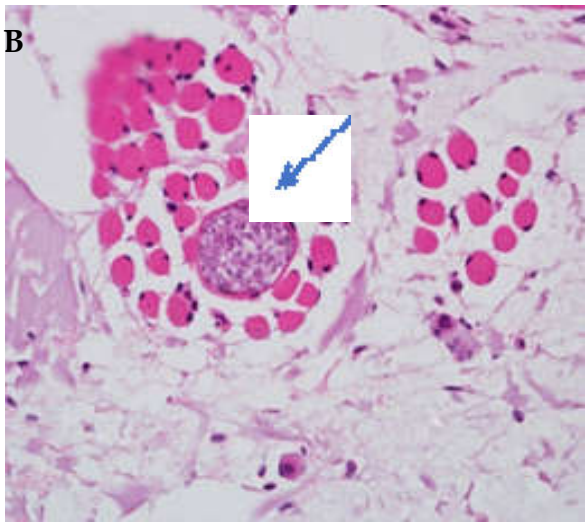
The prevalence of infection with *Sarcocystis* species in the animals sampled in all the locations during the wet season is shown in Table 1. Out of 1200 samples, 285 (23.8 %) were positive for *sarcocystis* infection. Pigs are the most infected with the parasite (30.57 %) compared with cattle and goats (21.39 %, 21.20 %). There was significant difference (P=0.004) in infection rates across the different animals.

The prevalence of Sarcocystosis in all tissues of animals across the various locations, is seen in figure 2. High infectivity was observed in skeletal muscle (29.2 %), and the least in diaphragm (4.8 %). The outcome was statistically significant (P=0.001).

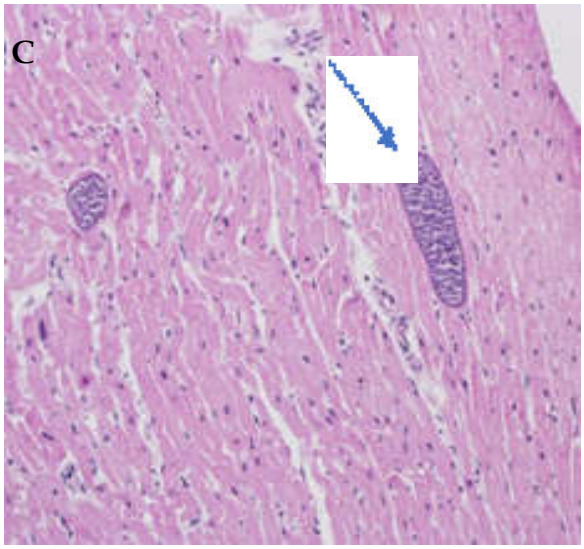
Table 2 shows Prevalence of sarcocystosis in cattle according to sex, age, tissue, breed and location. Infection is established in all cases and location is significantly associated with infection (P=0.000). Prevalence of infection in goats according to demographics is as presented in table 3. Infection is significantly association with tissue of goats (P=0.00). Table 4 indicates that *Sarcocystis* species infection is associated with location of the pigs (P=0.01).



(3a). Microcyst in oesophagus of Adult Female Female Cow in Otukpo



(3b). Microcyst in Tongue o f Adult Female Goat in Makurdi. H&E: ×400; 53×41 µm



(3c). Multiple Microcysts in Heart of Adult Male Pig in Makurdi

H&E; ×200; 141×48 to 44×31 µm

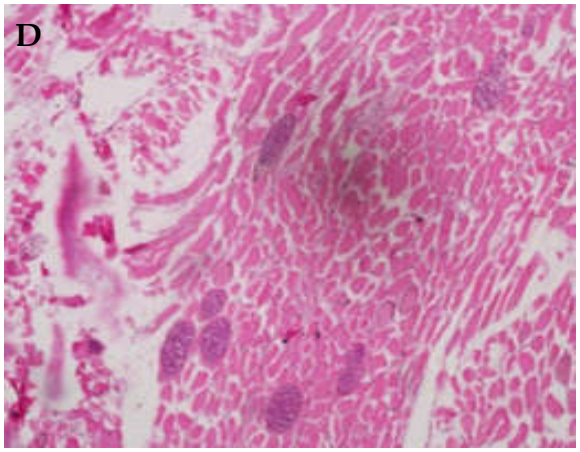
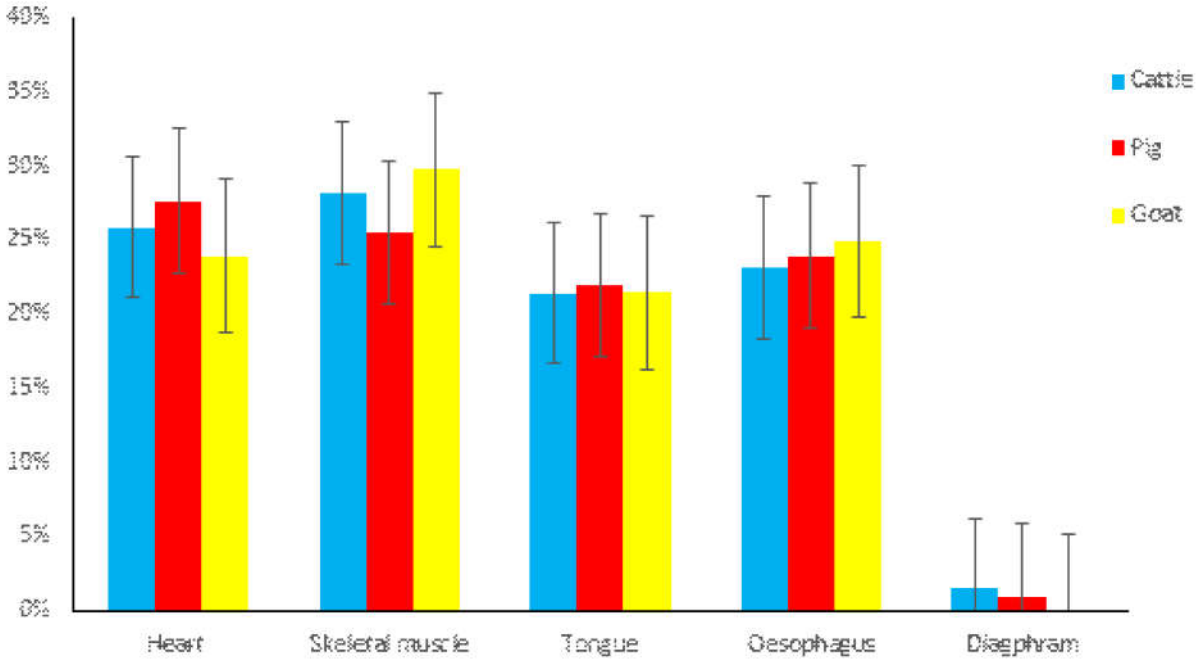


Plate 3d: Multiple Microcyst in Heart of Young Female Cow in Makurdi

H&E: ×400; 28×16 to 20×1 µm

Table 1: Overall prevalence of *Sarcocystis* species in animals in relation to age and sex

Type of Animal Cattle		No. Examined	No. Positive (%)	χ^2	P-value
Animal	Cattle	603	129 (21.4)	10.937	0.004*
	Goats	283	60 (21.2)		
	Pigs	314	96 (33.6)		
Sex	Male	434	102 (23.5)	0.023	0.88
	Female	766	183 (23.9)		
Age	Young	127	24 (18.9)	1.847	0.17
	Adult	1073	261 (24.3)		



χ^2 = 27.755; P = 0.000*

Figure 2: Median of Sarcocysts Intensity in Different Tissues of Ani-mals. Error bars represent 95% CI

Table 2: Prevalence of *Sarcocystis* species in Cattle in Benue State (Wet season)

Type of Animal Cattle		No. Examined	No. Positive (%)	χ^2	P-value
Location	Makurdi	351	98 (27.9)	25.09	0.000*
	Otukpo	203	30 (14.8)		
	(Adikpo	49	1 (2.0)		
Sex	Male	195	37 (19.0)	1.003	0.32
	Female	408	92 (22.6)		
Age	Young	41	9 (22.0)	0.008	0.93
	Adult	562	120 (21.4)		
Breed	SG	261	40(15.3)	10.074	0.002*
	WF	342	89(26.0)		
Tissues	Heart	158	37 (23.4)	7.98	0.09
	Muscle	161	41 (23.5)		
	Tongue	137	29 (21.2)		
	Oesophagus	131	22 (16.8)		
	Diaphragm	16	0		

Keys: SG- Sokoto Gudali; WF- White Fulani; No.- Number; j"^{2}- Chi-square; P-value- Probability value

Table 3: Prevalence of *Sarcocystis* species in Goats in Benue State in Wet Season

Type of Animal Goats		No. Examined	No. Positive (%)	χ^2	P-value
Location	Makurdi	146	32 (21.9)	2.905	0.23
	Otukpo	100	24 (24.0)		
	Adikpo	37	4 (10.8)		
Sex	Male	98	24 (24.5)	0.97	0.33
	Female	185	36 (19.5)		
Age	Young	40	5 (12.5)	2.111	0.15
	Adult	243	55 (22.6)		
Breed	AD	152	35(23.0)	0.655	0.42
	RS	131	25(19.1)		
Tissues	Heart	74	11 (14.9)	16.026	0.00*
	Muscle	83	22 (26.5)		
	Tongue	51	19 (37.3)		
	Oesophagus	75	8 (10.7)		
	Diaphragm	0	0		

Keys: RS- Red Sokoto; AD- African Dwarf; No.- Number; χ^2- Chi-square; P-value- Probability value

Table 4: Prevalence of *Sarcocystis* species in Pigs in Benue State in Wet Season

Pigs		No. Examined	No. +ve (%)	χ^2	P-value
Location	Makurdi	286	96 (33.6)	13.537	0.001**
	Otukpo	0	0		
	Adikpo	28	0		
Sex	Male	141	41 (29.1)	0.27	0.60
	Female	173	55 (31.8)		
Age	Young	46	10 (21.7)	1.982	0.16
	Adult	268	86 (32.1)		
Breed	L	161	43(26.7)	2.326	0.13
	P	153	53(34.6)		
Tissues	Heart	92	34 (37.0)	18.532	0.001**
	Muscle	81	32 (39.5)		
	Tongue	63	21 (33.3)		
	Esophagus	73	8 (11.0)		
	Diaphragm	5	1 (20.0)		

Keys: L-Local Breed; P-Pig White; No.- Number; x^2- Chi-square; P-value- Probability value

Discussion

The overall prevalence in this study was observed as 285 (23.8 %) out of 1200 tissues sampled each from 1200 animals, namely: cattle (n=603), goats (n=283) and pigs (n=314). As an overview of the status of sarcocystosis in Benue State, this prevalence can be considered high. Though moderate, compared to previous reports of near 100%, especially in cattle (Rahdar and Kardooni, 2017; Mavi *et al*., 2020) and from different parts of the world such as Brazil (Da Silva *et al*., 2002), USA/Argentina (More *et al*.,2011) and Egypt (Sayed *et al*., 2008).

In this study, one tissue of an organ from

each animal was sampled, this was also done by some authors, like Faghiri *et al*. (2019) who observed *Sarcocystis* species in at least one of the organs of 100 cattle samples diagnosed histologically. Also, this is based on the observations of other authors that animals infected with *Sarcocystis* species usually present macro/microcysts or both in tongue, oesophagus, heart, skeletal muscle or diaphragm (Gokpinar *et al*., 2014; Rahdar and Kardooni, 2017; Dong *et al*., 2018). Parasites, immediately after infection of the intermediate host, circulate in the blood stream and can be carried to different body parts before encystment (Gokpinar *et al*., 2014; Decker Franco *et al*., 2019). In the light of this, it was observed in this study that the skeletal muscle is the most infected tissue (figure 2) of the examined samples showing sarcocysts, while diaphragm was the least. This corroborates with intense infection found relatively often in muscles, esophagus and heart tissues and rarely in diaphragm (Januskevicius *et al*., 2017). Though in the present study, samples of diaphragm were not readily available hence the low rate observed. This however, does not agree with some authors who have reported up to 94 % in diaphragm compared to other tissues (Mohammed *et al*., 2020). Importantly, since only one tissue sample was obtained from each animal given that the distribution among tissues and organs was uneven, the percentages of infection shown in this report might have been underestimated.

On careful examination of meat before sampling, a few of the skeletal muscles of pork had macrocysts (12 out of 1200) and 5 of them were infected with both microcysts and macrocysts. There are not many reports of this nature in pork and up till the time of this report nothing is known about the macrocysts observed in pork destined for consumption in Benue State though very common. It is known that not all *Sarcocystis* species form macrocysts (Decker Franco *et al*., 2019) and is the observations by many authors in Nigeria (Kudi *et al*., 1991; Obijiaku *et al*., 2013; Mac *et al*., 2018) and around the world (Imre *et al*.,2019; Mohammed *et al*., 2020; Hamidi *et al*., 2020). Observations made in the present study corresponds to Barham *et al*. (2004), who observed high numbers of macrocysts in parts of goats during wet season. Macrocysts have mostly been observed in other animals such as and cattle (Faghiri *et al*., 2019); sheep (Minuzzi *et al*.,2019; Al-Saadi *et al*., 2020); South American camelids (Decker-Franco *et al*., 2018) and Old World carmels (Gareh *et al*., 2020).

The current study demonstrated that histological examination is effective in detecting *Sarcocystis* species, here, cysts can be seen in stained tissue sections with haematozylin and eosin. This has been carried out by many researchers most of which were later confirmed to be *Sarcocystis* species by molecular methods (Anderson *et al*., 1992; Faghiri *et al*., 2019; Metwally *et al*., 2020; Rudaitytė-Lukoðienė *et al*., 2020). Histological method is effective and can be used for an initial screening, nevertheless, the use of PCR in routine analysis of meat is recommended and is more sensitive and effective than other methods (Kamber *et al*., 2017).

In the present study, it was observed that factors conducive for higher prevalence of sarcocystosis could be more during the wet season. The period is characterized by water run-offs carrying oocysts, increased pasturing in the process associating more with intermediate hosts leading to high infectivity rate in animals. Data gathered during the period showed a significantly high

($P<0.05$) rate of infection. This agrees with Barham *et al*. (2004) who observed a similar trend of high micro and macrocyst infestation in goats during the wet season, attributing it to a period of closer association with definitive hosts, age of the animal and a transient period of low and high infection rate. In the present study area, it is observed that the wet season is a period when animals graze more which increases their probability of infection from contaminated pasture, during feeding, with sporulated sporocysts or oocysts due to their association with definitive hosts.

This study recorded a generally higher infection rates in pigs (33.6 %) compared to cattle and goats with significant difference

statistically (P=0.01). Other authors have reported such similar high rate up to 73.36 % due to reasons that have to do with breeding conditions, often leading to carcass condemnation in France (Avapal *et al*., 2004) and India (Chhabra and Samantaray, 2013). There are however contrary opinions about low infection rate of pigs compared with cattle in Lithuania (Januskevicius *et al*., 2017) and Philippines (Claveria *et al*., 2001).

Infection rate of *Sarcocystis* species in cattle and pig is significantly associated with location (P<0.05). This is an indication that location was a major determining factor for infection. Generally, unhygienic environment plays an important role in the effective transmission of *Sarcocystis* species (Romero *et al*., 2017), considering that dogs and humans can act as definitive hosts for some *Sarcocystis* species, this is similar to an observation made by Imre *et al*. (2019). In parts of Africa, especially Nigeria, open grazing has been in practice and has recently been banned due to clashes with farmers and community members. However, the ban implies minimal interactions of livestock with definitive hosts. Animal rearing involves movement of cattle from Northern parts of the country to Southern parts yearly, looking for and feeding on contaminated pastures, with lots of stray dogs around defecating without any form of control. These animals are also brought into the state from neighboring states/countries all through the year for commercial purposes, these are measures that encourage continuous transmission. Additionally, free access of wild carnivores to pastures can favor contamination with infective stages of the parasite. Based on this study, the prevalence of sarcocystosis in animals, deposes therefore, an environmental contamination of the parasites by infected carnivores. Sporocysts of sarcocysts are capable of resisting and retaining their infectivity in the environment despite factors, such as high temperature and some forms of disinfection for a long period of time (Dubey *et al*., 2016). The discrimination of *Sarcocystis* species should be considered of primary importance because, humans are definitive hosts of *S. hominis* and *S suihominis* with zoonotic risk for consumers of raw or undercooked meat (Dubey *et al*., 2015; Rahdar and Kardooni, 2017). On the other hand, sarcocystosis can lead to serious economic outcome as the parasite is considered one of the causes of Bovine Esinophilic Myositis (BEM), which is a specific inflammatory myopathy with lesions in striated muscle leading to carcass condemnation (Vangeel *et al*., 2007; Rubiola *et al*., 2020).

In conclusion, the rate of infection in this study (23.80 %) indicates that *Sarcocystis* species are present and widely distributed in cattle, goats and especially pigs slaughtered in the abattoirs at Otukpo, Adikpo and Makurdi in Benue State. Generally, sex, age and breed are not determining factors for infection (P>0.05), but predilection sites (tissues) and location of the animals were significantly associated with infection (P<0.05). Macroscopic sarcocysts were observed only in pigs. Microcysts seen were of high intensity in some animals and of two different shapes/ forms implying diversity due to developmental stage at the time of sampling and/or possible species difference. The tissues sampled; tongue, esophagus, heart, skeletal muscles and diaphragm harbored sarcocysts. Parasites observed are of zoonotic and public health importance.

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