Original Article

Prevalence of Hydatidosis and Characterization of *Echinococcus Granulosus* in Cattle, Goats and Swine in Benue State, Nigeria

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Abstract

Hydatidosis is a serious zoonotic disease caused by Echinococcus granulosus species complex. This research was conducted to determine the prevalence of infection of the disease in Benue State, Nigeria. The study area was divided into three (3) locations: Makurdi, Otukpo and Adikpo abattoirs. The carcass of each animal which included Cattle, goats and pigs was inspected carefully for the presence of hydatid cysts and infection. The organs infected and the numbers of cysts were recorded. Animal sex and age were also recorded. Hydatid cysts collected were preserved in 70% ethanol and transported to the laboratory for analysis. In the laboratory, cysts sizes were measured, microscopic examination of hydatid fluid was performed to determine cysts fertility and Haematoxylin and eosin staining technique was performed. Overall prevalence was 30.86% (679/2200), infection rates in the sampling sites were significant (P<0.05), with the lungs being the most infected organ (42.36%), followed by the liver (19.36%), while mixed infections involving the liver and the lungs were detected in 2.91% of the livestock sampled. The cysts were examined under the microscope to determine fertility, out of the 1296 hydatid cysts collected and examined, (52.40%) of the hydatid cysts were fertile, (35.80%) were sterile while (11.80%) were calcified. Lung cysts were found to be more fertile (64.27%) compared to liver cysts (35.73%). Prevalence based on age was statistically significant (P<0.05) with adult more infected (33.01%) than the young (21.88%). Infection rate was seen to be more during the wet season (34.38%) than during the dry season (25.78%). There was a direct relationship between age, number and size of hydatid cysts as the number and size of the cysts increase with increase in age of the animal. The outcome of this study justifies the need for comprehensive intervention in hydatidosis management and prevention.

Key words: Hydatidosis, *Echinococcus granulosus*, Benue State.

OPEN ACCESS

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Specialty Section:*This article was submitted to Sciences section of NAPAS.*
Submitted date: *24th Dec., 2022*
Accepted date: 28th March,2023
Published date:

Citation: Okoh, M.E., Omudu, E.A., Atu, B.O., Obadiah, H.I *(2023)* Prevalence of Hydatidosis and Characterization of *Echinococcus Granulosus* in Cattle, Goats and Swine in Benue State, Nigeria - *Nigerian Annals of Pure & AppliedSciences. 6(1):42-51 DOI:10.5281/zenodo.7338397*

Publisher:*cPrint,Nig.Ltd*
Email:*cprintpublisher@gmail.com*

AccessCode

Introduction

Hydatidosis is a zoonotic disease caused by larval stages of cestodes belonging to the genus *Echinococcus*. It is characterised by long term growth of the metacestode(hydatid cysts) in the intermediate host. It is a wide spread infection throughout the world and occurs in all domestic livestock including camels, cattle, goats, pigs and sheep. (Okolugbo *et al*., 2014) . Hydatidosis has recently been included by the World Health Organization (WHO) as a neglected tropical disease(Ahmed *et al*., 2018). According to WHO, 1 million people around the world are suffering from Cystic Echinococcosis , and an estimated burden of 88,082 - 1,590,846 disability- adjusted life years(DALYs) has been attributed to Echincoccosis.The annual monetary burden of the disease due to treatment costs and hydatidosis related livestock losses has been estimated at US $ 3 billion(WHO 2019).

According to Craig *et al*., 2007, Human cystic echinococcosis (hydatid disease) continues to be a substantial cause of morbidity and mortality in many parts of the world and so there is need for early diagnosis of *echinococcosis* to enable effective prevention and control of the disease.Benue State is a transit area for cattle moving from North to South and they are continously mixing with the local breed which can bring about the spread of this disease. This work is therefore aimed at determining the prevalence of hydatidosis in cattle, goats and swine in Benue state**.**

MATERIALS AND METHODS

Study site

Seven major abattoirs were sampled which are: North bank, Wurukum, Modern market, Wadata, Adikpo, and Otukpo (Eupi and Ole-ena) abattoirs. These are located within zone A, B and C senatorial districts of Benue State, North-Central zone of Nigeria.

Figure 1: Map of Benue State Showing study areas
(AbattoirSource: Ministry of Lands, Survey and Solid minerals Makurdi (2022)

Study design and Sample size

The study was carried out from October 2019 to March 2021 to determine the level of infection of hydatidosis in cattle, goats and swine in Benue State. The sample size was calculated using a formula described by Thrusfield (2005), using a prevalence of 46.2% based on a previous study by Okolugbo *et al.* (2013).

$$N=Z^2 p (1-p) / d^2$$

Where:

N is the minimum sample size,
Z is the confidence interval which is the range of values in which the population mean is likely to be maintained with a given level of probability, defined by the standard errors of the sampling distribution,
p is the expected frequency of the condition of interest and
d^2 is the inverse of 95% allowable error which is the desired precision, expected frequency is 46.2%.

Therefore substituting the values in the formula above; for sample size (n)

$$n = \frac{1.96^2 \times 0.462 \times 0.538}{0.05^2}$$

$$n = \frac{0.9548527296}{0.0025}$$

n= 381.94 (Approximately 400 samples).

Field sample collection

Ante Mortem Investigation

The abattoirs which are key facilities for slaughter were visited. A total of 2,200 livestock were examined visually and physically. Information about the date of the slaughter, the total number of cattle slaughtered per day at the slaughter house, source of water and method of disposal of offal was recorded. The age of the livestock was determined based on eruption and wear of deciduous and permanent teeth. (Keuroghlian *et al.*, 2010) the sex of the livestock was also recorded.

Post Mortem Investigation

Post slaughter examination, thorough examination of the livers and lungs was done through inspection, palpation and incision of the slaughtered cattle. Small portions of liver and lung was collected transversely with a knife from each examined, and was placed in a well labeled plastic bags for further examination in the laboratory. All hydatid cysts were thus collected and sent to the laboratory for parasitological examination. Lungs and liver were taken and preserved in 70% ethanol in clean plastic containers and transported to the laboratory for analysis. The fertility of the cysts were determined and recorded based on the criteria established by Manyuele, (2011). Cysts with protoscolices were considered as fertile cysts, while fluid filled cysts without protoscolices were considered as sterile cysts and solid and sand contained cysts were considered as calcified cysts.

Histological Examination

In the histology unit, there were a number of distinct steps that were involved in producing histological slides, ready for analysis. Some of the steps are described below:

The tissues collected were fixed in 10% formalin for 48hours to prevent the tissues from further degenerating before reaching the histology laboratory. Small portions of the tissues were dissected and sectioned gently into a labeled cassette and closed. Dehydration was done in series of graded concentration of Methanol (70 %, 90 % and 100%) respectively for period of 12 hours. The tissue samples were cleared in xylene for 2 hours. The tissues were placed into paraffin wax that was divided in three places (wax 1, 2 and 3) for a period of 1 hour each. The tissues were removed from the cassette and placed into an embedding wax. The solid blocks of molten paraffin wax were placed on a wooden block to hold the wax containing tissues firm when placed on a microtome machine while sectioning. The tissues were sliced into sections ranging from 5-10 microns using a microtome machine. The tissues were folded into folded ribbons. Water was added into a floating bath and heated up to a temperature of 50 - 55°C, the folded tissues were placed on the warm

water so as to unfold the sliced section. Using a well labeled slide a section of tissue was picked and allowed to dry in order for the tissue to stick onto the slide, Gareh *et al*., (2020).

Staining (Haematoxylin and Eosin Stains) Technique.

The sections were dewaxed in xylene for 3-10 minutes, the sections were passed through 100%, 90% and 70% alcohol for 1 minute each and were washed in water. The sections were stained in Ehrlich Haematoxylin for 10 minutes and washed with water. The sections were differentiated using 1% acid alcohol for 1 minute and washed with water. The sections were placed in Scott tap water for 2 minutes which gives the sections a blue color and wash with water. The sections were stained in Eosin for 2 minutes and were washed with water. The sections were dehydrated using ascending grades of alcohol from 70% to 90% and 100% alcohol for 1 minute each. The sections were cleared in Xylene. Sections were mounted with cover slip using a DPX moundant (Dibutylphthalate, Polystyrene Xylene) which preserves stains and the slides containing the sections were dried quickly, Gareh *et al*., (2020).

Microscopic Examination

After histological examination of the tissues, the slides were viewed under a light microscope, starting with the X10 magnification and then the X40 magnification for a clear view. Pictures of positive samples were taken and recorded.

Data Analysis

The data collected included the number of cysts, cysts size, sex of livestock, age of livestock as well as variables like location of the cyst overall infection rate was calculated by taking the total number of infected livestock divided by the total number of livestock examined and multiplied by 100. Infection and age of the livestock, infection and sex of the livestock , infection and sampling site, Infection and predilection site, Infection and livestock examined were compared using Chi- squared (x^2). Infection rate in each sampling site was calculated in percentage by taking the total number of livestock infected in each site divided by the total number of surveyed livestock in each sampling site multiplied by 100. Abundance of cyst and infection rate was compared using T- test while Cyst size and infection rate and cyst fertility with infection rate were compared using analysis of variance (ANOVA). The test was kept at $p<0.05$ significance level

Results

Prevalence of hydatidosis in the different livestock examined based on location and seasonality is detailed in Table 1. Adikpo had the highest prevalence at 47.50% while Otukpo had the least at 19.33%. According to the livestock examined, goat was the most infected at 50.50% and pig had the least prevalence at 14.00. There was a significant difference at $p<0.05$. Table 2 showed the prevalence of Hydatidosis based on gender of the livestock examined. Male was found to be more infected (37.92%) than female (33.16%). It was statistically insignificant. Prevalence of hydatidosis based on Age of life stock was detailed in Table 3. Adults were more infected (36.90%) than young (28.70). There was a significant difference at $p< 0.05$.

Table 4 showed the prevalence of hydatidosis based on predilection site. Lungs had a prevalence of 24.11% and liver was 10.78%. It was statistically significant. The abundance of Cysts in live stock was detailed in Table 5 .Goats had the most cyst count(59.43%)while pigs had the least(14.86%). Cyst fertility is shown in Table 6, and lungs had more fertile cysts (74%) than liver(26%). Abundance of Cysts in relation to age is represented in Table 7. Correlation was significant as the rate of infection was more in Adults than young live stock examined.

Table 1: Prevalence of hydatidosis in the locations sampled in cattle, Goats and Swine at different seasonalities

		Dry Season			Wet Season			Total			
Location	**Livestock**	**No. Examined**	**No. Positive**	**Prevalence**	**No. Examined**	**No. Positive**	**Prevalence**	**No. Examined**	**No. Positive**	**Prevalence**	x^2
Makurdi	Cattle	200	50	25%	400	114	29%	600	164	27%	
	Goat	100	18	18%	100	28	28%	200	46	23%	
	Swine	100	20	20%	100	32	32%	200	52	26%	
Otukpo	Cattle	100	25	25%	200	86	43%	300	111	37%	19.816
	Goat	100	22	22%	100	26	26%	200	48	24%	
	Swine	50	23	46%	50	18	36%	100	41	41%	4.642[LS]
Adikpo	Cattle	100	27	27%	100	38	38%	200	65	33%	18.463
	Goat	50	24	48%	100	35	35%	150	59	39%	
	Swine	100	23	23%	150	70	47%	250	93	37%	
Total		**900**	**232**	**26%**	**1300**	**447**	**34%**	**2200**	**679**	**30.86%**	

Table 2: Prevalence of *hydatidosis* based on gender

Sex	**No. Examined**	**No. Positive**	**Prevalence**	x^2	**P-value**
Male	1024	409	39.94%	73.981	0.000*
Female	1176	270	22.96%		
Total	**2200**	**679**	**30.86%**		

Table 3: Abundance of cysts in relation to age of livestock

Age of Livestock	**Number Infected**	**Cyst Count**	**R**	**p-value**
Adult	586	986		
Young	93	310	1.000	0.000**
Total	**682**	**1296**		

Table 4: Prevalence of *hydatidosis* based on predilection site.

Organs	**No. Examined**	**No. Positive**	**Prevalence**	**x²**	**P-value**
Liver	1100	213	19.36%		
Lungs	1100	466	42.36%	819.781	0.000*
Both	2200	64	2.91%		
Total	4400	743	16.89%		

Table 5: Abundance of *hydatid cysts* in affected livestock.

Livestock	**Organs**	**Cyst Count**	**Occurrence (%)**	**F**	**P-value**
Cattle	Liver, Lungs	589	45.45		
Goat	Liver, Lungs	427	32.95	4.841	0.040*
Swine	Liver, Lungs	280	21.60		
Total		1296	100.00		

Table 6: Cyst fertility among infected Organs

Organs	Fertile Cyst	Sterile Cyst	Calcified Cyst	Total	F	P-value
Lungs	466	278	89	833(64.27%)	1.091	0.355
Liver	213	186	64	463(35.73%)		
Total	679	464	153	1296(100)		

Table 7: Abundance of cysts in relation to age of livestock

Age of Livestock	Number Infected	Cyst Count	R	p-value
Adult	586	986		
Young	93	310	1.000	0.000**
Total	**682**	**1296**		

****. Correlation is significant at the 0.01 level (2-tailed)**

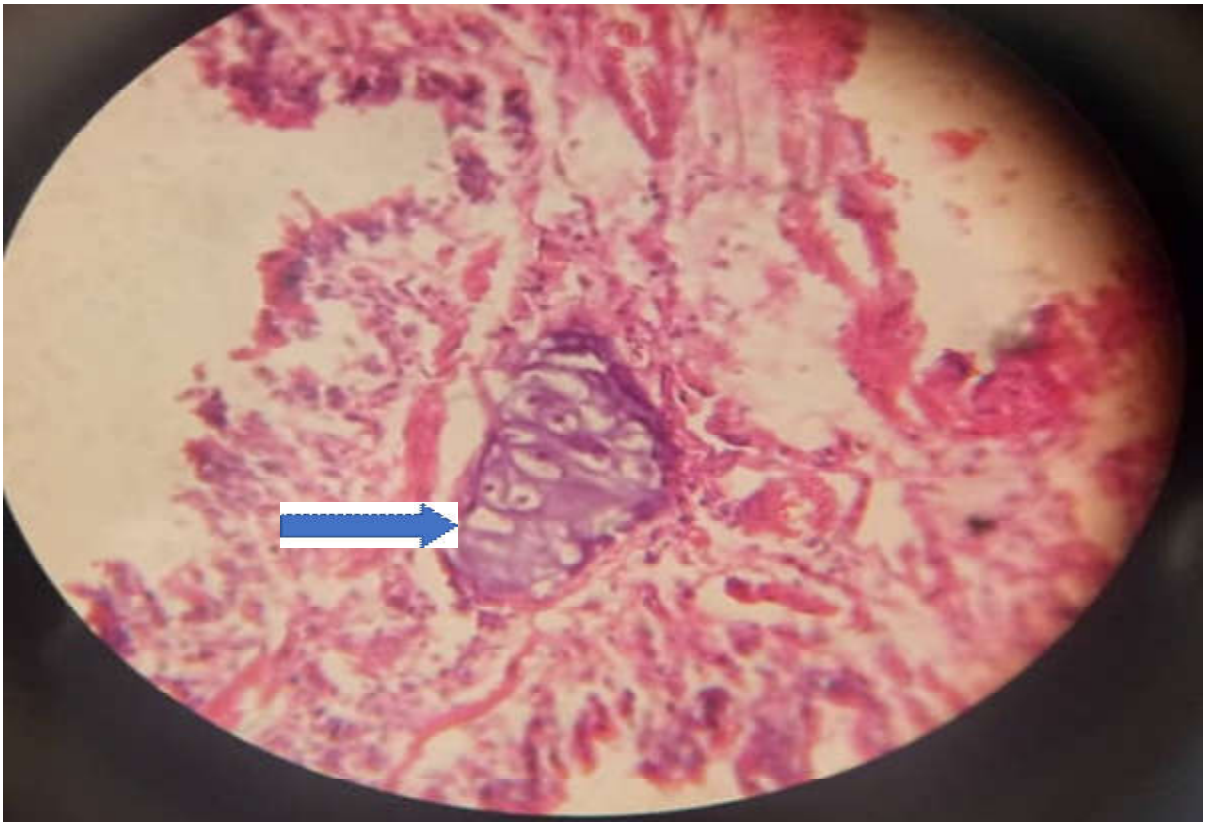

Figure 2: Hydatid cyst in lungs of an infected cow as shown in the arrow

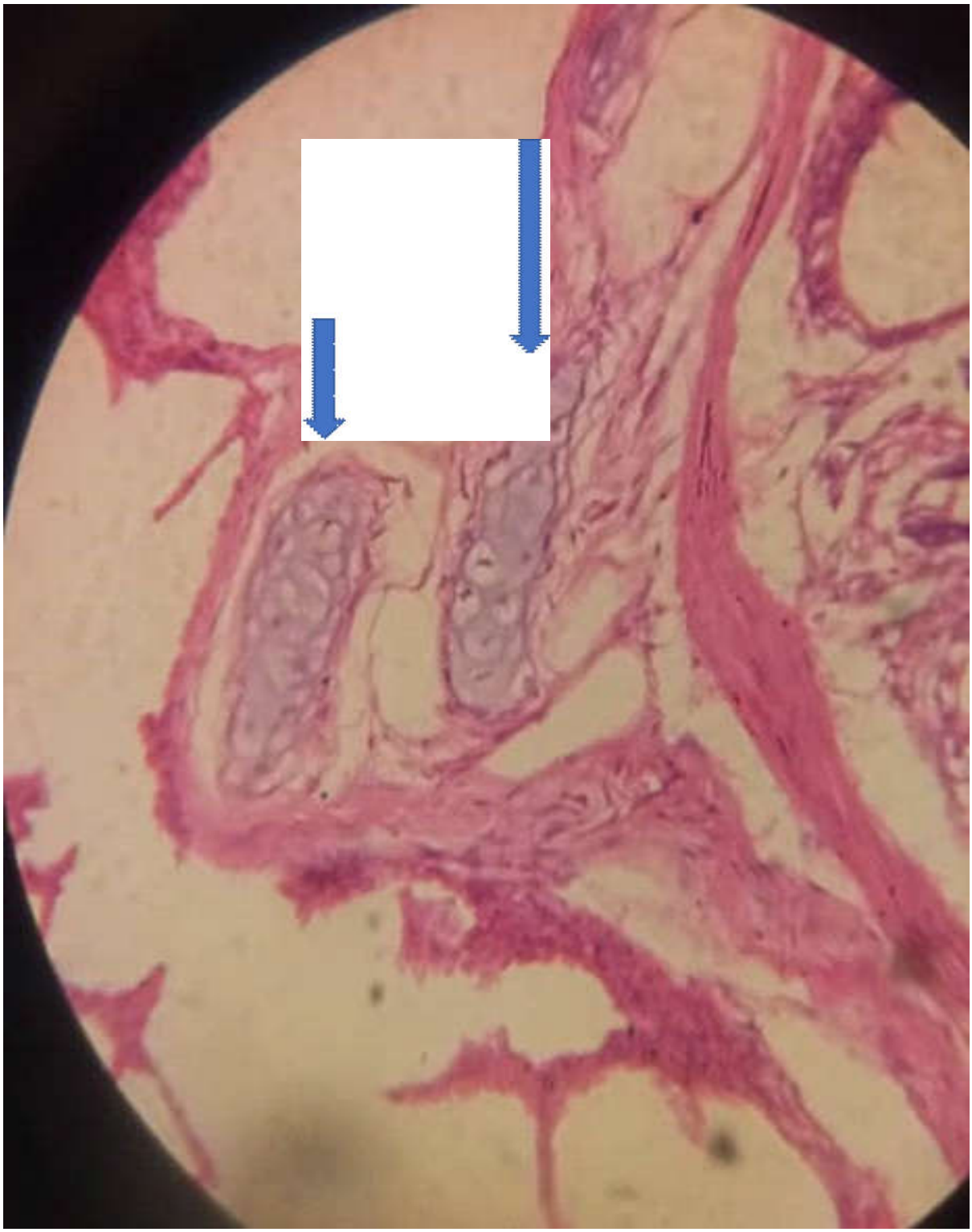

Figure 3: Liver of goat with hydatid cyst as shown by the arrow

Discussion

This study revealed an overall prevalence of 34.88% for hydatidosis in Benue State. This result is in close agreement with the following: 38.20% (Haleem *et al.*,2018) in South Eastern Ethiopia, 34.05% (Kebede *et al.*,2009) from Bahir Dar and 30.00% (Kamali *et al.*,2018) in Iran. However, in the present study it is lower than the findings of Okulogbo *et al.* (2013) who recorded 46.2% in Sokoto Nigeria, Addy *et al.* Reported a

prevalence of 53.1% in kenya (2012) and Ahmed *et al*. (2018) recorded 88.00% in sudan .

The present study was higher than some studies done by Jegede *et al*.(2010) in Kano, Nigeria who reported a prevalence of 26.2%, Mekuria *et al*. (2015) who reported a finding of 24.5% in Ethiopia, Ochi *et al*. (2015) recorded 3.99% in Sudan and Singh *et al*. (2012) who reported 1.59% in India. The variation in the different reports of prevalence in hydatidosis can be attributed to the different environmental conditions which could affect the transmission of hydatidosis, Other variables like difference in culture and lack of proper disposal of infected animals could contribute to the difference in prevalence.

In this study, the relatively high prevalence could be as a result of the close proximity between dogs and the domestic animals observed in the abbatoirs which encourages the propagation of the infection. The prevalence of hydatidosis varied significantly ($p< 0.05$) in the different locations sampled. Makurdi had a prevalence of (40.25%), Otukpo (19.33%) and Adikpo had a prevalence of (47.50%). This is in line with a study done at Asella munincipal in Ethiopia by Haleem *et al*.(2018) who recorded different prevalence from different parts of Ethiopia. Nekemte in western Ethiopia had 36.66% prevalence while Hawasa, Southern Ethiopia had 15.9% prevalence. The difference in the prevalence in this study could be as a result of differences in cultural practices, the availability of animals to be slaughtered in the different abattoirs and also the close proximity observed between the animals and dogs which is the definitive host. The prevalence of hydatidosis based on the livestock slaughtered is statistically significant ($p<0.05$) Cattle (35.00%), Goats (50.50%) and Pigs(14.00%). This is in agreement with a study done in Iran by Azami *et al*.(2013) were the highest prevalence of hydatidosis was observed In sheep (47.7%) then cattle (25.8%), and Toulah *et al.* (2012) and this study is in contrast with a report given by Addy *et al.* (2012) where Cattle was found to have a higher prevalence(25.8%) than sheep(16.5%) and goat(10.8%) .

The possible reason for the difference in the prevalence of the infection may be as a result of the contact between the final hosts faeces and the livestock which are the intermediate hosts;

Another reason could be that there is a favourable environment for sustaining the life cycle of the parasite.

The prevalence of hydatidosis varied significantly ($p<0.05$) with age. This is in line with studies by Akol *et al*.(2015), Haleem *et al*.(2018), Mekuria *et al*.(2015) ,Okulogbo *et al*.(2013)and many others.

This could be because older animals have longer exposure to eggs of *Echinococcus granulosus* hence increased chance of acquiring the infection. Studies conducted by Ibrahim *et al*.(2009) and Kebede *et al*.(2009) suggest that prevalence of hydatidosis is heavily influenced by age.

Sex based prevalence show no statistical difference ($p>0.05$) in agreement with Akol *et al*. (2015). This may be because of similar physiology in both sexes.

Lungs of the livestock were more affected than liver in this study ($p<0.05$). This is in line with Studies done by Haleem *et al*. (2018), Mekuria *et al*.(2015) and in contrast to studies conducted by addy *et al*. (2012) and Kamali *et al.* (2018) where the liver of infected animals were more affected.

The reason being that the lungs have large capillary beds encountered by the blood borne migrating *Echinococcus* onchospheres which is in agreement with Kebede *et al*. (2009)

The fertility rate of the cysts in the lungs (74.00%) was higher than that of the liver(26.00%). It is line with a study done by Azami *et al*. (2014) where fertile cysts were 77% and 33% in lungs and cattle respectively

This may be due to the softer consistency of the lung tissues that allows easier development of the cyst(Haleem *et al*., 2018)

Conclusion

In this study, it has been found that Hydatidosis is prevalent in Benue State Nigeria.

A prevalence of 34.88% which is high

compared to results of previous studies conducted in other Parts of Nigeria .

This is an indication that the disease will continue to be of medical and veterinary importance both in humans and livestock if no control measures are put in place.

A higher proportion of hydatid cysts collected being fertile (74.00 %) imply that the nature of the cyst is an important factor that can affect stability of *E. granulosus* parasite.

Acknowledgment

We are most grateful to the laboratory technician Mr. Peter of the histology unit, Biotechnology centre at Ahmadu Bello University Zaria for his expertise in preparing histology slides for this work. We are also grateful to our undergraduate project students who helped with sample collection from the abattoirs. May GOD bless you all.

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