Fasciola infection in goats slaughtered from Port Harcourt metropolis Rivers State, Nigeria

A. E. Abah¹, G. N. Wokem² and I. I. Sounyo¹

 Department of Animal and Environmental Biology, Faculty of Science, University of Port Harcourt, PMB 5323 Port Harcourt 50001, Rivers State, Nigeria; 2. Department of Medical Laboratory Sciences, Rivers State University of Science and Technology, Nkpolu-Oroworukwo, Port Harcourt 50001, Rivers State, Nigeria. Corresponding author: A. E. Abah, e-mail: austin.abah@uniport.edu.ng Co-authors: GNW: wokem.gloria@ust.edu.ng, IIS: itsounyo06@gmail.com Received: 10-04-2019, Accepted: 28-06-2019, Published online: 03-08-2019

doi: 10.14202/IJOH.2019.76-80 **How to cite this article:** Abah AE, Wokem GN, Sounyo II. *Fasciola* infection in goats slaughtered from Port Harcourt metropolis Rivers State, Nigeria. Int J One Health 2019;5:76-80.

Abstract

Background and Aim: Fascioliasis is a zoonotic disease of public importance that affects animals and man. This study was aimed at assessing *Fasciola* infection among goats slaughtered in Port Harcourt metropolis.

Materials and Methods: A total of 323 gallbladder and fecal samples were collected from three different abattoirs (Mile 3, Rumuokwurusi, and Trans-Amadi) between September 2015 and June 2016 and analyzed using standard parasitological method.

Results: Of the 323 fecal samples, 20 (6.19%) were found to be positive for *Fasciola* egg. Mile 3 abattoir 17 (5.26%) had higher prevalence than Trans-Amadi abattoir 3(0.93%) while Rumuokwurusi had no infection (χ^2 =0.11; p=0.001). Gallbladder infection was 5.57%. Trans-Amadi abattoir had the highest prevalence of 11(3.41%) followed by Mile 3 abattoir 7(2.17%) and Rumuokwurusi abattoir had none (χ^2 =0.91; p=0.339). Infection based on gender showed that 5.88% of female animals and 0.31% of males were ova positive. At Trans-Amadi, 0.93% of females against 0.0% of males were infected (p>0.05). At Mile 3, it was 4.95% of females against 0.31% of males (p=0.001). Rumuokwurusi abattoir had no infection. Gallbladder infection showed Trans-Amadi 2.79% of females and 0.62% of males; Mile 3, 2.17% of females and 0.0% of males and Rumuokwurusi had no infection.

Conclusion: *Fasciola* infection is prevalent in goats in Port Harcourt. The health implication of these findings is that humans are at risk as fascioliasis is a zoonotic disease. There is a need to intensify effort to create more awareness of the possible consequence of this infection.

Keywords: fascioliasis, goats, infection, Nigeria, prevalence, zoonotic disease.

Introduction

Fascioliasis is a zoonotic disease caused by *Fasciola* spp. *Fasciola hepatica* and *Fasciola gigantica* are responsible for this infection [1]. *F. hepatica* is the species of concern in Europe, even though the distributions of both overlap each other in several areas in Asia and Africa [2]. According to the World Health Organization (WHO), human fascioliasis is on the priority list of neglected tropical diseases (NTD) [3].

Fascioliasis has been described as a rising foodborne disease [4-6] and is said to affect almost 56 million people [7]. *Fasciola* which is a significant parasite of man in several countries usually infects cattle, sheep, and other herbivores. Incidentally, the meats from these animals which are definitive host of the parasite [8] constitute the main source of protein for people in Nigeria.

Geographical distribution of fascioliasis is determined by presence and distribution of *Lymnaea*

Copyright: Abah, *et al.* This article is an open access article distributed under the terms of the Creative Commons Attribution 4.0 International License (http://creativecommons.org/licenses/ by/4.0/), which permits unrestricted use, distribution, and reproduction in any medium, provided you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license, and indicate if changes were made. The Creative Commons.org/ public Domain Dedication waiver (http:// creativecommons.org/ publicdomain/zero/1.0/) applies to the data made available in this article, unless otherwise stated.

species, the intermediate hosts of parasite, and conditions such as climate, weather pattern, and increased rainfall. Poverty also promotes the prevalence of the infection in endemic areas [6]. Aliyu *et al.* [9] reported the first occurrence of fascioliasis in Nigeria which resulted in the death of 3000 goats in Borno Province. The worth of liver loss was colossal and was all time unequal for an individual abattoir above a period of 3 years [10]. Their assessment showed that the 36 states including the Federal Capital Territory will show similar losses in not less than one abattoir per state [9].

Studies in Nigeria have reported incidences of fascioliasis mostly in cattle and sheep [11-15], but there is a paucity of information on goat and especially in Southern Nigeria. This study was aimed at assessing *Fasciola* infection among goats slaughtered in Port Harcourt metropolis, Nigeria.

Materials and Methods

Ethical approval

Ethical clearance was obtained from the Research Ethics Committee of the University of Port Harcourt. Consent was also sought from the Veterinary Section, Ministry of Agriculture, Rivers State, before samples were collected.

Study area

There are many abattoirs in Port Harcourt City. Rivers State, but most of them are not officially recognized by Rivers State Government. However, Trans-Amadi abattoir which was chosen as one of the study areas is among the officially recognized ones in the state. Trans-Amadi abattoir is the main abattoir in the city of Port Harcourt: it is located in Trans-Amadi Industrial Area. Trans-Amadi lies at 4°48'53" N latitude and 7°2'14" E longitude (Figure-1), is a thousand-hectare industrial area situated in an area that supports a strong manufacturing sector and is considered to be a major industrial zone in Obio-Akpor Local Government Area, Rivers State [16]. Trans-Amadi abattoir is the largest abattoir in the Niger Delta. Rumuokwurusi lies at 4°51'6"N, 7°2'653"E, latitude and longitude. It is located on the outskirt of Port Harcourt, close to Port Harcourt-Aba expressway and Rivers State College of Art and Science. Mile 3 lies at 4°47'24"N latitude and longitude 6°59'36"E. It is one of the three extensions of Diobu. It is a densely populated neighborhood of Port Harcourt, Rivers State located within Port Harcourt metropolis. This neighborhood is rank among the most commercially lively places in the city.

Study population

The study population was male and female goats from the Trans-Amadi, Rumuokwurusi and Mile 3 abattoirs all in Obio-Akpor Local Government Area, Rivers State situated in the Southern part of Nigeria. The goats were brought mainly from the Northern part of Nigeria (Kano, Sokoto, and Nasarawa states), as well as from Chad Republic. The samples were



Figure-1: Map of Port Harcourt City Local Government Area and its environs showing sample locations (Source: Cartography Department, University of Port Harcourt).

selected based on random sampling; a total of 323 goats were sampled.

Sample size determination and design

The sample size for the study was determined by taking the estimated prevalence of 30% fascioliasis using the formula given by Cochran, 1963 [17]. They were 323 goats investigated in this present study.

$$n = \frac{t^2 \times P(1-P)}{M^2}$$
$$n = \frac{1.962^2 \times P(1-P)}{M^2}$$

Where.

n = Required sample size

t = Confidence level of 95% (standard value of 1.96)

P = Estimated prevalence = 30%

M = Margin of error at 5% (standard value of 0.05) Hence,

 $M^2 = 0.05$ and p = 0.3 (30%). A total of 323 animals were sampled.

Sample collection

The abattoirs were visited between 7 am and 9 am thrice weekly (Mondays, Wednesdays, and Fridays). Samples of feces and gallbladder were collected from randomly selected goats in each of the abattoirs. Fresh fecal materials were collected directly from the rectum of slaughtered animals using a pair of sterile hand gloves. The samples were placed in clean labeled bottles containing 10% formalin and transferred to the parasitology laboratory of Animal and Environmental Biology, University of Port Harcourt for examination for eggs of F. gigantica, F. hepatica, and other intestinal parasites following the WHO standard [18]. The sex and sources of each randomly selected goat were noted and recorded. Adult flukes seen were removed, measured, and recorded. The content of gallbladder was emptied into a clear sterile bottle containing 10% formal saline and later examined for various stages and species of *Fasciola* as described by Cheesbrough [19].

Laboratory examination

Formol-ether concentration technique for fecal samples

This was as described by Arora and Brij [20]. Four ml of a well-mixed stool sample was put in a tube containing 4 ml of 10% formalin. The suspension was sieved using a coffee strainer into a centrifuge tube. The filtrate was centrifuged at 2000 rpm for 2 min. The supernatant was discarded and the sediment resuspended in 10 ml of physiological saline. The sediment was suspended in 7 ml of formal saline, after which 3 ml of ether was added. The tube was closed with a stopper and shaken vigorously. The stopper was removed and the tube centrifuged at 2000 rpm for 2 min. Four layers became visible: The top layer of ether, the second layer of plugs of debris, the third layer of formalin, and the fourth layer of sediment. The plug of debris was detached from the side of the tube with the aid of a glass rod and the liquid was discarded leaving a small amount of formal saline for resuspending the sediment. A little was transferred to a clean glass slide at a time, covered with a coverslip and examined under the microscope at $10 \times$ and $40 \times$ objectives, respectively, to view for eggs and this was repeated until the whole sediment was examined.

Identification of Fasciola eggs and other intestinal parasites

The *Fasciola* ova were identified with their characterized large, oval, and yellowish color with a distinct flat operculum, which were seen as described by other researchers [15,18,19]. Furthermore, species identification of the recovered *Fasciola* from postmortem specimen of the livers collected was based on the morphological features of the parasites according to differential keys [15,18,19].

Statistical analysis

Data obtained were statistically analyzed using the Statistical Package for the Social Sciences (SPSS) version 21 (IBM, USA).Categorical data were presented in the form of frequencies and percentages (%). Chi-square test was used to test for association or differences between categorical variables. $p\leq0.05$ was considered statistically significant.

Results

In fecal samples, 6.19% were positive for *Fasciola* infection. The prevalence of the parasite seemed to be significantly different among the three study areas (Table-1). The prevalence rate in Mile 3 abattoir (5.26%) was statistically higher than Trans-Amadi abattoir (0.93%) while Rumuokwurusi had no infection (χ^2 =10.11; p=0.001).

Overall, the prevalence of 5.57% was detected in gallbladder samples. The prevalence of infection was significantly different within the three studied areas. Trans-Amadi abattoir statistically, had the highest prevalence of ova in gallbladder specimens (3.41%) followed by Mile 3 abattoir (2.17%) and Rumuokwurusi abattoir had none (χ^2 =0.91; p=0.339).

A total of 5.88% of female and 0.31% of males were positive (Table-2). At Trans-Amadi abattoir, Fasciola ova in fecal specimen were isolated from female goats. At Mile 3 abattoir, Fasciola ova in fecal sample of female goats were higher 4.95% than male goats 0.31%, the difference in proportion was statistically significant with $\chi^2=13.59$; p=0.001. Rumuokwurusi abattoir had no infection. Of 293 female goats investigated, 4.95% were infected while 0.62% of 30 males were infected. At Trans-Amadi abattoir, Fasciola infection prevalence in gallbladder sample of female goats was 2.79% and male goat was 0.62%. There was a significant difference (χ^2 =4.53; p=0.03). At Mile 3 abattoir, the infection rate in gallbladder samples of female goats was 2.17% while male goats were 0.0%. This difference in proportion was statistically significant (χ^2 =5.20; p=0.02). Rumuokwurusi abattoir had no infection (Table-3).

In fecal specimens, the overall infection rate was 6.19% *Fasciola* spp., 8.36% Hookworm, 1.86% *Trichostrongylus* spp., 1.86% *Strongyloides* spp., and 1.24% *Coccidia* oocysts (Table-4).

Discussion

From the abattoirs in Port Harcourt, fecal specimens showed that *Fasciola* infection was prevalent in two of the abattoirs, Mile 3 and Trans-Amadi abattoirs with an overall prevalence of 6.19%. This

Table-1: Prevalence of Fasciola gigantica ova in fecal and gallbladder samples from the three locations in Port Harcourt.

| Abattoir location | Fecal sample (n=323) | | Gallbladde | χ² (p-value) | |
|-------------------|------------------------|------------------------|------------------------|------------------------|---------------|
| | Number examined (%) | Number infected (%) | Number examined (%) | Number infected (%) | |
| Trans-Amadi | 135 (41.79) | 3 (0.93) | 135 (41.80) | 11 (3.41) | 4.67 (0.03)* |
| Rumuokwurusi | 68 (21.05) | 0 (0.0) | 68 (21.05) | 0 (0.0) | Not derivable |
| Mile 3 | 120 (37.15) | 17 (5.26) | 120 (37.15) | 7 (2.17) | 4.33 (0.04)* |
| Total | 323 (100.0) | 20 (6.19) | 323 (100.0) | 18 (5.57) | () |
| χ² (p-value) | | 10.11 (0.001)* | | 0.91 (0.339) | |

*Statistically significant (p<0.05)

Table-2: Prevalence of infection among sexes from fecal sample in the three locations in Port Harcourt.

| Abattoir location | Male goats (n=30) | | Female goats (n=293) | | Total (n=323) | χ² (p-value) |
|-------------------------|------------------------|------------------------|------------------------|------------------------|---------------|----------------|
| | Number examined (%) | Number infected (%) | Number examined (%) | Number infected (%) | | |
| Trans-Amadi | 19 (5.88) | 0 (0.0) | 116 (38.39) | 3 (0.93) | 135 (41.80) | 1.34 (0.247) |
| Rumuokwurusi | 6 (1.86) | 0 (0.0) | 62 (19.20) | 0 (0.0) | 68 (21.05) | Not derivable |
| Mile 3 | 5 (1.55) | 1 (0.31) | 115 (36.22) | 16 (4.95) | 120 (37.15) | 13.59 (0.001)* |
| Total | 30 (9.29) | 1 (0.31) | 293 (90.71) | 19 (5.88) | 323 (100.0) | (<i>, ,</i> |
| χ^2 (p-value) | | Not derivable | | 9.16 (0.002)* | | |
| *Chabieries III. eienif | icont (n <0.05) | | | | | |

*Statistically significant (p<0.05)

| Table-3: Prevalence of infection among sexes from gallbladder sample in the three locations in Port Ha | rcourt. |
|--|---------|
|--|---------|

| Abattoir location | Male (n=30) | | Female (n=293) | | Total (n=323) | χ² (p-value) | |
|-------------------|------------------------|------------------------|------------------------|------------------------|---------------------------------------|---------------|--|
| | Number examined (%) | Number infected (%) | Number examined (%) | Number infected (%) | | | |
| Trans-Amadi | 19 (5.88) | 2 (0.62) | 116 (35.89) | 9 (2.79) | 135 (41.80) | 4.53 (0.03)* | |
| Rumuokwurusi | 6 (1.86) | 0(0.0) | 62 (19.20) | 0 (0.0) | 68 (21.05) | Not derivable | |
| Mile 3 | 5 (1.55) | 0 (0.0) | 115 (35.60) | 7 (2.17) | 120 (37.15) | 5.20 (0.02)* | |
| Total | 30 (9.29) | 2 (0.62) | 293 (90.71) | 16 (4.95) | 323 (100.0) | | |
| χ² (p-value) | . , | 0.50 (0.479) | × / | 0.26 (0.612) | , , , , , , , , , , , , , , , , , , , | | |

*Statistically significant (p<0.05)

Table-4: Percentage prevalence of Fasciola spp. and other intestinal parasites from goat fecal samples in Port Harcourt.

| Abattoir location | Total number examined | Number (%) of the prevalence of intestinal parasites | | | | | |
|----------------------|--------------------------|--|------------|--------------------------|-----------------------|------------------------|--|
| | | Fasciola spp. | Hookworm | Trichostrongylus spp. | Strongyloides spp. | <i>Coccidia</i> oocyst | |
| Trans-Amadi | 135 | 3 (2.22) | 17 (12.59) | 5 (3.70) | 2 (1.48) | 4 (2.96) | |
| Rumuokwurusi | 68 | 0 (0.00) | 3 (4.41) | 1 (1.47) | 1 (1.47) | 0 (0.00) | |
| Mile 3 | 120 | 17 (14.17) | 7 (5.83) | 0 (0.00) | 3 (2.5) | 0 (0.00) | |
| Total (%) | 323 | 20 (6.19) | 27 (8.36) | 6 (1.86) | 6 (1.86) | 4 (1.24) | |

preponderance was lower than 9% reported in a similar study in Zaria [21] and higher than 2% in Pakistan [22]. The present result is comparable with those obtained in Ethiopia [23] where 6.8% was reported in young goat. This low prevalence could be attributed to the level of consciousness and awareness by the management of the Abattoirs, if not, the climatic condition, moisture, and swampy nature of Port Harcourt is favorable for both the snail intermediate host and the parasite survival and transmission.

The results obtained from the three selected abattoirs for gallbladder samples showed that fascioliasis was prevalent in the same two abattoirs (Trans-Amadi and Mile 3) with an overall prevalence of 5.57%. This is similar to what was obtained from the fecal samples 6.19%. There was no statistical difference between the two infection rates. The observed prevalence of 5.57% in the study was in line with the result of livestock in Borno state, Nigeria [24], where 5.7% found in their study on liver fluke (*Fasciola* and *Dicrocoelium hospes* eggs) infections.

The result obtained also showed variations between prevalence in fecal and gallbladder samples within the study area. The prevalence of infection from gallbladder specimens was 3.4% which was statistically higher than prevalence from fecal samples of 0.93% in Trans-Amadi abattoir. This is in agreement with the findings of Ibrahim et al. [25]; in their study, on bovine fascioliasis in Ethiopia, where the prevalence rate was recorded to be higher in organs (liver, 12.4%) than from fecal specimen (2.43%) for F. gigantica. This lower prevalence in fecal samples has been attributed to intermittent nature of expulsion of eggs through the feces [25-27]. It could be equally attributed to the tubular nature of gallbladder which could restrict easy flow of laid eggs out of the site; hence, the fluke dwell and lay eggs there. The results obtained from Mile 3 abattoir from fecal 5.26% and

gallbladder samples 2.17% did not agree with their findings because there was a statistically significant difference between the two infection rates (p<0.05).

The present study also revealed that female goats harbored more infection than male goats. The overall prevalence obtained from this study showed 5.88% for female goats and 0.31% for male goats from fecal samples while 4.95% for female goats and 0.62% for male goats from gallbladder specimens. This result is similar to the observations obtained in Maiduguri, Nigeria [28] where female goats had a higher prevalence rate 0.47% than male goats 0.26% as well as a similar report from Zaria [21]. They attributed their findings to an exhibited increased susceptibility to helminthosis associated with hormonal activity, especially at pregnancy [28] which was attributed to immune suppressive effect of reproductive hormones of the female animals during pregnancy and lactation period [21]. However, in this present study, more female goats were available for sampling in the abattoirs selected as they do not slaughter young female goats, and very old ones with reduced reproductive hormones. The young female goats are naturally allowed to stay for propagation.

The present study showed the prevalence of some intestinal helminths such as hookworm, *Trichostrongylus* spp., *Strongyloides* spp., and *Coccidia* oocyst. This is similar to Esonu [21], who also observed some gastrointestinal parasites, for example, *Strongyloides* spp. and *Coccidia* oocyst among others. The tropical climatic condition of the study area is suitable for the transmission of these parasites and hence may be the reason for their presence.

The implication of this finding is that humans are at risk of the zoonotic infection as fascioliasis is a trematode zoonosis and is classified as an NTD which affects humans. Some *Trichostrongylus* worms can incidentally infect humans if they ingest water or vegetables contaminated with infective larvae, which happen in regions with poor sanitary conditions. Human infections with *Trichostrongylus* have been reported. *Strongyloides* spp., especially *S. stercoralis*, is considered zoonotic and human coccidiosis has been reported.

Conclusion

Fascioliasis and other parasitic helminth infections are prevalent in goats slaughtered in Port Harcourt abattoirs. The health implication of these findings is that humans are at risk of fascioliasis and some of helminthic zoonosis. There is a need to intensify effort to create more awareness of the knowledge of these parasites, pasture management, and anthelmintics administration to curb the spread and possible consequence of this infection.

Authors' Contributions

GNW, AEA, and IIS designed the work, IIS collected and analyzed samples, and supervised by GNW and AEA. AEA prepared the manuscript. All authors read and approved the final manuscript.

Acknowledgments

The authors are grateful to the authorities of Veterinary Section, Ministry of Agriculture, Rivers State and the management of the abattoirs for giving us access to conduct the study.

Competing Interests

The authors declare that they have no competing interests.

Publisher's Note

Veterinary World (Publisher of International Journal of One Health) remains neutral with regard to jurisdictional claims in published map and institutional affiliation.

References

- Mas-Coma S, Bargues MD, Valero MA. Fascioliasis. In: Bruschi F, editor. Helminth Infections and their Impact on Global Public Health. Vienna: Springer; 2014. p. 93-122.
- 2. Anosike JC, Opara MN, Okoli CG, Okoli IC. Prevalence of parasitic helminths among-ruminants in Etiti area of Imo state, Nigeria. Anim Prod Res Adv 2005;1:13-9.
- 3. World Health Organization. Fascioliasis, Foodborne Trematode Infections. Geneva, Switzerland: World Health Organization Headquarters; 2018.
- Freites A, Colmenares C, Alarcón-Noya B, García ME, Díaz-Suárez O. Human fascioliasis in mara municipality, Zulia state. Venezuela: Prevalence and asociated factors. Invest Clin 2009;50:497-506.
- 5. Karahocagil MK, Akdeniz H, Sunnetcioglu M, Cicek M, Mete R, Akman N. A familial outbreak of fascioliasis in Eastern Anatolia: A report with review of the literature. Acta Trop 2011;118:177-83.
- 6. Mera y Sierra R, Agramunt VH, Cuervo P, Mas-Coma S. Human fascioliasis in Argentina: Retrospective overview, critical analysis and baseline for future research. Parasit Vectors 2011;4:104.
- World Health Organization. Fact Sheet on Foodborne Trematode Infections. Media Centre. Fact Sheet No. 368; Updated May 2015. Available from: http://www.who.int/mediacentre/factsheets/fs368/en/. Last assessed on 20-10-2018.

- Olusegun-Joseph TS, Doherty VF, Edeghagha BO. Prevalence of fascioliosis in cattle slaughtered at Zaria abattoir. J Environ Iss 2011;1:50-6.
- 9. Aliyu AA, Ajogi IA, Ajanusi OJ, Reuben RC. Epidemiological studies of *Fasciola gigantica* in cattle in Zaria, Nigeria using coprology and serology. J Publ Health Epidemiol 2014;6:85-91.
- World Bank. (2018) World Bank List of Economic; July 2006. Available from: http://www.iscb.org. [Last accessed on 2018 Mar 20].
- Njoku-Tony RF. Bovine fascioliasis among slaughtered cattle in selected abattoirs in Imo state, Nigeria. World Rural Obs 2011;3(1) 59-63
- 12. Njoku-Tony RF, Okoli GC. Prevalence of fascioliasis among slaughter sheep in selected abattoirs in Imo state, Nigeria. J Am Sci 2011;7:361-6.
- Ardo MB, Aliyara YH, Lawal H. Prevalence of bovine fascioliasis in major abattoirs of Adamawa state Nigeria. Bay J Pur Appl Sci 2013;6:12-6.
- 14. Shitta KB. Gastro-intestinal helminths of slaughtered cattle at Wukari abattoir Taraba state, North-Eastern Nigeria. Int J Epidemiol Infect 2013;1:15-8.
- Ngele KK, Ibe E. Prevalence of fasciolopsis in cattle slaughtered at Eke market abattoir, Afikpo, Ebonyi state, Nigeria. Anim Res Int 2014;11:1958-63.
- 16. Encyclopædia Britannica. Port Harcourt (Nigeria). www. britannica.com. Last accessed on 18-05-2018.
- Israel GD. Sampling the Evidence of Extension Program Impact. Program Evaluation and Organizational Development, IFAS, University of Florida; 1992. p. 1-5.
- World Health Organization. Evaluation of certain contaminants in food. World Health Organ Tech Rep Ser 2017; (1004):1-66.
- Cheesbrough M. Parasitological testing. In: District Laboratory Practice in Tropical Countries. Part 1. 2nd ed. Cambridge: Cambridge University Press; 2005. p. 196-8, 223-5.
- Arora DR, Brij BA. Medical Parasitology. 3rd ed. Darya Gani, New Delhi, New York: CBS Publishers and Distributors Pvt., Ltd.; 2010. p. 158-9.
- 21. Esonu DO. Prevalence of *Fasciola gigantica* eggs in sheep and goats and on salad vegetables in Zaria and environs, Nigeria. A Thesis Submitted to the School of Postgraduate Studies, Ahmadu Bello University, Zaria for the Award of the Degree of Master of Science in Veterinary Public Health and Preventive Medicine; 2014. p. 32-63.
- 22. Shahzad W, Mehmood K, Munir R, Waqar A, Muhammad I, Rashid A, *et al.* Prevalence and molecular diagnosis of *Fasciola hepatica* in sheep and goats in different districts of Punjab, Pakistan. Pak Vet J 2012;32:535-8.
- 23. Lakem Z, Alebie A. A study on the prevalence, risk factors and financial loss of cattle, sheep and goats fascioliasis in Debrezeit town, Ethiopia. Am Eur J Sci Res 2015;10:126-33.
- Nwosu CO, Srivastava GC. Liver fluke infections in livestock in Borno state, Nigeria. Vet Q 1993;15:182-3.
- 25. Ibrahim N, Wasihun P, Tolosa T. Prevalence of bovine fascioliasis and economic importance due to liver condemnation at Kombolcha Industrial Abattoir, Ethiopia. Int J Vet Med 2009;8(2):1-7.
- 26. Sánchez-Andrade R, Paz-Silva A, Suárez JL, Panadero R, Pedreira J, López C, *et al.* Influence of age and breed on natural bovine fascioliasis in an endemic area (Galicia, NW Spain). Vet Res Commun 2002;26:361-70.
- 27. Hillyer GV. Immuno diagnosis of human and animal fascioliasis. In: Dalton J, editor. Fascioliasis, 13. Wallingford, Oxon, UK: CAB Inter; 1999. p. 435-47.
- 28. Mbaye AW, Shingu P, Luka JA. Retrospective study on the prevalence of *Fasciola* infection in sheep and goats at slaughter and associated economic losses from condemnation of infected liver in Maiduguri abattoir, Nigeria. Niger Vet J 2010;31:224-8.
