

Liverfluke Coprological Cross-Sectional Survey in Cattle, Sheep and Goats in Sharazur District Kurdistan- Iraq

Kwestan Najm Ali^{1*}, Hardi Fattah Marif¹, Nawroz Akram Kakarash² and Hawsar Othman Mohammed³

¹ Department of Internal Medicine and Clinic, College of Veterinary Medicine, University of Sulaimani, Sulaimani City, Kurdistan Region, Northern Iraq.

² Department of Anatomy and Pathology, College of Veterinary Medicine, University of Sulaimani, Sulaimani City, Kurdistan Region, Northern Iraq.

³ Department of Basic Sciences, College of Veterinary Medicine, University of Sulaimani, Sulaimani City, Kurdistan Region, Northern Iraq.

*Corresponding author: E-mail address: kwestan.ali@univsul.edu.iq, Phone number: +964-07701598448

Doi: <https://doi.org/10.37940/AJVS.2021.14.1.3>

Received: 16/2/2021 Revised: 21/4/2021 Accepted:4/5/2021

This article is licensed under a CC BY (Creative Commons Attribution 4.0)

<http://creativecommons.org/licenses/by/4.0/>.

Abstract

Cross-sectional coprological survey was conducted to know the prevalence of liver flukes in cattle, sheep and goats in Sharazur district Kurdistan- Iraq from June 2018 to March 2020. Parasitological examination of fecal samples collected from 685 animals from several field (280 sheep, 245 goats and 160 cattle) was done by using sedimentation method (Fecal Egg Count Reduction Test- FECRT). We revealed that an overall *Fasciola* species prevalence were (49.48%). Liver fascioliasis was documented highly in sheep (55.71%), followed by cattle (47.5 %) and goats (43.67%). Risk factors such as age and sex showed a significant effects on the prevalence of liver flukes ($P < 0.05$). A higher prevalence rate was noticed and identified in animals older than 3 years old (57.66%) and it was higher than those found in middle age (47.71%) and in young animals (31%). The prevalence of female Fascioliasis was (52.74%) and higher than male Fascioliasis which was (40%).

Keywords: *Fasciola gigantica*, *Fasciola hepatica*, Foodborne diseases, Snails, Sharazur district.

مسح مقطعي لذبائح الكبد في الأبقار والأغنام والماعز في منطقة شارزور كردستان العراق

الخلاصة

أجريت دراسة مقطعية لتحديد مدى انتشار حلزون الكبد في الأبقار والأغنام والماعز في منطقة شارزور في كردستان العراق من حزيران 2018 الى اذار 2020. الفحص الطفيلي لعينات براز تم جمعها من 685 حيواناً من عدة حقول (280 نعجة ، 245 ماعز و 160 بقرة) باستخدام طريقة الترسيب (اختبار تقليل عدد البويضات - FECRT). اظهرت الدراسة ان نسبة الاصابه الكليه كانت (49.48%). بينت الدراسة ان اعلى نسبة اصابه كانت في الأغنام (55.71%) ، تليها الأبقار (47.5%) والماعز (43.67%). اظهرت عوامل الخطر مثل الجنس والعمر تأثيراً كبيراً على انتشار عدوى حلزون الكبد ($P > 0.05$). لوحظ أن اعلى نسبة انتشار كانت في الحيوانات الأكبر من 3 سنوات (57.66%) بينما سجلت الحيوانات متوسطة العمر نسبة اصابه (47.71%) وفي الحيوانات الصغيرة (31%). كان انتشار داء المتورقات أعلى في إناث الحيوانات (52.74%) منه في ذكور الحيوانات (40%).

Introduction

Fascioliasis is one of the crucial food-borne parasitic diseases in the class trematoda. It is one of the group of neglected atropical diseases (NTDs). According to the WHO roadmap for minizing the public health problems of NTDs, Food-borne trematodiasis are one of the 17 NTDs included (1). It is also known as the most widely distributed disease among zoonotic diseases, and it has a great impact on economic loses in animal industry in the world especially in cattle and sheep. This parasitic disease is very common in ruminants, particularly in cattle, sheep, buffaloes, swine and goats and also in human beings (2). The causative agent is the endoparasitic trematodes of the genus *Fasciola* (3). lives in the bile ducts (4). The two most common species of liver flukes are *Fasciola hepatica* and *F. gigantica*. They can cause hepatobiliary system infection in cattle and sheep, and also have an impact on public health. It has been documented that *F. gigantica* is causing liver fluke nfection in tropical areas whereas, *F. hepatica* is mostly reported in temperate climates (3). *F. gigantica* is found in Asian and African countries. While, *F. hepatica* found worldwide. Moreover, *F. hepatica* is the main cuase of human fascioliasis and it has recently been reported as an emerging and re-emerging zoonotic disease in many countries (5). This disease has been stated as a significant disease in livestock-rearing areas, beacuse it

has major effects on the animal husbandry industries (6). Recently climate change increase Fascioliasis in livestock animals globally, because it can messuer as s contributing factor for the disease burden. This can be expalied by wetter summers and warmer winters as well as supporting the larger population of intermediate host (mud snail). Climate change can also has an impact on disease management (sheep treatment only, cattle will not be treated and veterinary interaction limitation). Climate change may cuase treatment and chemical control resistance and moving livestock animals in the absence of commercial vaccines (7). Abdominal pain is one of the most common clinical signs of the parasite infections. This occures bacuase of the movement of the young parastite within the liver and the bile ducts. Extensive damage of the liver is occurring because of this migration and may cause portal cirrhosis, fever, nausea, vomiting, hepatomegaly, hepatic tenderness, and eosinophilia are also common. (8, 9). Lowered weight gain, anemia, reduced animal productivity, reduced milk yiled and meat production and lowered feed conversion efficiency are seen in the infected animlas (10). Diagnosis can be difficult without clear clinical manifestations. *Fasciola* is diagnosed by Fecal Egg Count Reduction Test (FECRT), detecting antibody in serum and milk and feces. (11, 12, 13). Imaging techniques, such as computed tomography (CT) and ultrasonography (US), endoscopic retrograde

cholangiopancreatography (ERCP) and sphincterotomy have been used for diagnosis (14, 15, 16). The most effective and widely used antihelmintic for the treatment of fascioliasis in animals is triclabendazole (TCBZ). This drug is highly effective for mature and immature stages of the parasite (17). It has been recommended by CDC that TCBZ is the first line agent that used for the treatment of fascioliasis in humans, and this may have some side-effects such as dizziness, fever and abdominal pain one week after the using of the treatment (18). Other drugs, such as pain killers, can also be used to treat some symptoms such as pain and diarrhea. Surgical operation may be necessary in very few cases where cholangitis, and bile duct infection in the liver has developed. The aims of the present study are the establishment the prevalence rate of fascioliasis in cattle, sheep and goats in Sharazur district Kurdistan Region-Iraq. Moreover, to investigate the possible effects of sex and species on the prevalence of fascioliasis among sampled ruminants.

Materials and Methods:

2.1. Study area

The present study was carried out in (656 KM²), located in the Eastern of Sulaimani city. The area is mostly flatty and some parts has either hilly or mountainous and extends from (450-600 M) above the sea level. There is only one lake and many rivers in the area. The area

is also characterized with high humidity and high water fall with about (500-700 mm) yearly. The rain usually starts from the early autumn to the late spring. Weather is very dry in summers and rainy in winters which is a Mediterranean climate.

2.2. Sample sizes and sample distribution

The samples were taken from 10 ovine farms (fecal samples from 280 animals), 9 caprine farms (fecal samples from 245 animals) and 7 bovine farms (fecal samples from 160 animals) in all parts of the study area from June 2018 to March 2020. Samples were taken randomly from 510 female and 175 male animals from three different groups of age (100 samples < 1 year, 285 samples 1-3 years and 300 > 3 year). All farms were selected to be equally distributed in the study area. For this reason we used Google map to divide the study area. It is important to say that there were many bovine, caprine and ovine pasturing farms. Samples were directly sent to the Laboratory of the Veterinary Teaching Hospital for testing.

2.3. Laboratory procedure

Fecal Egg Count Reduction Test (FECRT), a sedimentation method was used to determine the numbers of fluke eggs/ gram of feces. The samples were mixed well and 10g of feces was weighed out and mixed with a little water in a 500ml beaker. The beaker was then top up with water. Three sieves (38 μ m, 150 μ m and 500 μ m)

were stacked with the smallest aperture at the bottom and largest at the top. The fecal water was slowly passed through the sieves followed by thorough washing with water until the water was run clear from the bottom sieve. The 500 μ m sieve was removed and washed through the remaining two sieves were repeated. The 150 μ m sieve was removed and the retentive on the surface of the 38 μ m sieve washed and the remaining contents backwash into a 500ml beaker. The beaker was topped up with water and left to stand for 4 minutes. The supernatant was poured off leaving approximately 100ml of sediment and then the beaker was refilled with water and left to stand for a further 4 minutes. This process was repeated until the supernatant was cleared. When clear the supernatant poured off to 100ml or less if possible without losing any sediment and the remaining contents transferred into a large square Petri dish. Two drops of Methylene blue was added and the number of *F. hepatica* eggs counted using a dissecting microscope. Results were given in the number of eggs/ gram of feces calculated was calculated by dividing the total number of eggs by 10. (19)

2.4. Statistical analysis

Variables were introduced into the statistical analysis were age and sex in three different animal species (sheep, goats and cattle) using SPSS v24, M.S. Excel (for graphs). The differences were considered statistically

significant when $P < 0.05$ with 95% confidence intervals (CI). The differences between age and sex of the different animal species were compared statistically using two-way analysis of variance, followed by post hoc (LSD).

Results and Discussion

Among the 685 fecal samples in all animal species with different ages and sex tested for liver fluke eggs 339 showed positivity, leading to an overall of 49.48% (95% CI). The percent positivity of liver fluke varied significantly ($p < 0.05$) among all species: 55.71%, 43.67% and 47.5 % in sheep, goats and cattle respectively (Table1). In addition, the statistical analysis showed that age and sex affected the percent positivity of liver fluke infection in all animal species. Positivity in percentage was higher in females (52.74%) (95% CI) than in males (40%) (95% CI) ($p < 0.05$) (Figure1). Moreover, percent positivity in animals older than 3 years old (57.66) was significantly ($p < 0.05$) higher than those in middle age (47.71%) and in young animals (31%) as shown in (Table1).

Table 1: Prevalence the infection rate of *Fasciola* spp among sheep, goats and cattle according to sex and ages by Faecal Egg Count Reduction Test (FECRT).

Factor	Animals	Sample size	Number of positive samples	Percent positivity %
Species	Sheep	280	156	55.71
	Goats	245	107	43.67
	Cattle	160	76	47.5
sex	Male	175	70	40
	Female	510	269	52.74
Age (year)	< 1year	100	31	31
	1-3 year	285	136	47.71
	> 3 year	300	172	57.33

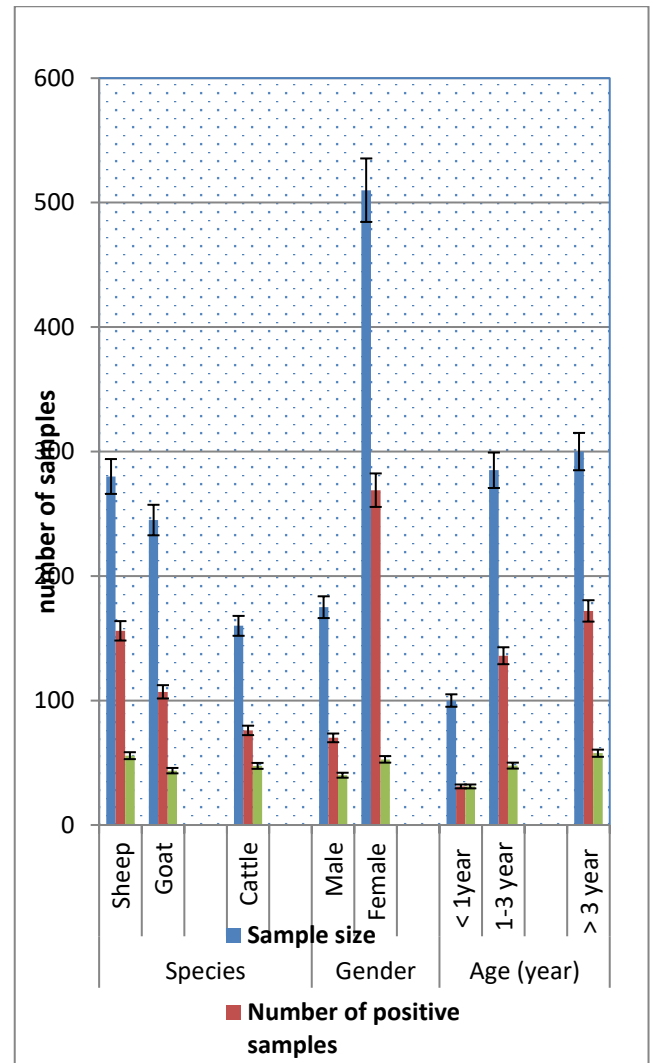


Figure 1: Total number of samples tested by fecal egg count reduction test (FECRT) in three different species of animal with sex and ages (year). Error bars indicate the 95% confident intervals.

Fascioliasis is considered to be a major issue in public health specially for farmers and veterinarians globally. This because of the great impacts on the animal products. Furthermore, it has been reported that anthelmintic treatment is not always effective because of the development of the drug resistance (17, 20). Risk factor identification of the fluke infection may cause the progression and development of

a good control strategies and treatment protocol measures for minimizing of the fluke infection this will cause the effectiveness of the animal production. (21).

In Iraq, many factors affect the distribution of the fascioliasis in various parts of the country, such as old-style and not standard animal agriculture and the great populations of the snail. Fascioliasis deemed to be one of the crucial health problem in the study area. It has economical and social influences of the people in Iraq (22).

The rate of the fluke infection in the current study in Sharazur district Kurdistan region-Iraq were high (49.48%), this may be due to that the area is very wet and has many rivers. The management system of the livestock animals and using water resources directly from rivers can also be considered as the potential factor of the flukes life cycle (23, 24). Moreover, the disease is chronic and still there is no control program by local authorities in Kurdistan Region for fascioliasis. The result is higher than that previously reported by (25) in Sindh Province of Pakistan (42.06%), (26), (25.46%) in Punjab-Pakistan, (27), (37%) in Zimbabwe, (28), (26%) in Kenya, (29), (46%) in Zambia, (30), (14.7%) in Egypt, (31), (10.92%) in Malang District – East Java, (32), (23.18 %) in Ireland, (33), (5.8%) in Batu City- Indonesia, (34), (0.87%) in Al- Najaf-Iraq, (35), (0.50%) in Kirkuk-Iraq, (36), (1.7%) in Kermanshah-Iran, (37), (3.28%) in Kashan-Iran, and (38),

(0.56%) in Arak- Iran. Moreover the result is lower than that previously documented by (39) (65%) in Ireland.

According to the species of animals the result showed that sheep had a highest prevalence rate which was (55.71%), to the disease than cattle (47.5%) and goat (43.67%), this was in agreement with the data obtained by (40) in sheep (7.1%) and goats were (3.9%) respectively, (41) in sheep (19%), cattle (17.8%), goat (11.5%), respectively, and (42) in sheep (5.7%) and goat (1.6%). The prevalence in sheep is high. This may be because of the animal grazing habits. Goats are mostly grazing on trees which is free from flukes however, sheep eating plants on the ground, where full of fluke infective stage (43, 44, 45 and 46). This is what researchers investigate in Morocco (47) and in Argentina (48). Availability of suitable habitat for snails as intermediate hosts, temperature and humidity are the main factors to consider in the epidemiology of fasciolosis (49).

The age has great effect ($p < 0.05$) on the rate of propagation of the disease. The prevalence of the disease is elevated with the age of the animals, the infection rate of *Fasciola* was the highest above 3 years (57.66%) while it was (47.71%) in 1-3 years age, and lowest in under 1 year age animals which was (31%). The high rate in the prevalence of this parasite with the age has been documented by (50). This may be due to the reason that animals do not develop

resistance against fluke infection with the increment of age (51). Research carried out by (52) stated that clinical signs and lesions in the liver can mostly be seen in the animals with old ages because they have more exposure to the parasite infective stage (metacercaria) (52). This can be explained by young animals are mostly stayed around the farms and feed in door, so that they have less chances to be exposed with the fluke metecercaria compared to adults (53).

In the present study we confirmed that the animal sex has significant effects ($p < 0.05$) on the occurrence of fascioliasis in the study area which is female has greater chance of affected by fascioliasis than male 269 (52.74% and 70 (40%) respectively. This study finding is in line with reports of (54) was recorded (39.7%) and (16.9%) in female and male animals, respectively, (55), (51.09%) and (50.39%) was recorded in female and male animals respectively, (56), (70.6%) and (36.06%) was recorded in female and male animals respectively. (57) who documented a higher prevalence in females (41.3%) than males (13.8%) and by (58) in females (46.99%) and males (19.96%). This may be due to the fact that females have pregnancy and lactation stress as well as spending more time in the pasture for grazing. (59). Many intrinsic risk factors such as genetics, physiology, and immunology and extrinsic risk factors such as environment and management practices present between male and female cattle. Hormonal and stress

influences are considered to have effects on the higher percentage of the fluke infection in the females as stress will lead to immune-suppression (57).

Conclusion

The current findings showed that fascioliasis was more common and has major impacts on economical losses in animal industry. This parasitic disease is mainly affecting ruminants in the study area. Hence, treatment and control strategies should be designed to reduce the prevalence liver flukes. This study also gives data for the further monitoring protocol in the treatment and prevention of the fluke infection in the region. Some factors associated with the prevalence of fascioliasis were noticed, including age, sex, and species of the infected animals.

Conflict of Interest

The authors disclose no conflicts related to the present study.

Acknowledgments

Authors are appreciating the director of the Veterinary Teaching Hospital and the Diagnostic Laboratory staff for their support and providing facilities to conduct this work.

References

1. FAO/WHO. Multicriteria-based ranking for risk management of food-borne parasites. 2014: 287

2. Abass KS, Ibrahim EK, Khalaf RN, Esmail RH. Prevalence of Liver Fluke Infections in Slaughtered Animals in Kirkuk Province Iraq. *J. Anim. Sci. Livest. Prod.* 2014; Vol. 2. No.2: 05.
3. Parkinson M, O'Neill SM, Dalton JP. Endemic human fasciolosis in the Bolivian Altiplano". *Epidemiol Infect.* 2007; Vol. 135. No. 4: 669-674.
4. Abraham JT, Jude IB. Fascioliasis in cattle and goat slaughtered at calabar Abattoirs. *J. Biol. Agric. Healthc.* 2014; Vol. 4. No. 18: 34-41.
5. Marcos LA, Maco V, Samalvides F, Terashima A, Espinoza J, Gotuzzo E. Risk factors for *Fasciola hepatica* infection in children: a case-control study. *Trans. R Soc. Trop. Med. Hyg.* 2006; Vol. 100 No. 2: 158-166.
6. McCann CM, Baylis M, Williams DJ. The development of linear regression models using environmental variables to explain the spatial distribution of *Fasciola hepatica* infection in dairy herds in England and Wales. *Int. J. Parasitol.* 2010; 40(9): 1021-1028.
7. LaCourse EJ, Perally S, Morphew RM, Moxon JV, Prescott M, Dowling DJ, O'Neill SM, Kipar A, Hetzel U, Hoey E, Zafra R, Buffoni L, Arevalo JP, Brophy PM. The Sigma Class Glutathione Transferase from the Liver Fluke *Fasciola hepatica*. *PLoS. Negl. Trop. Dis.* 2012; 6(5): e1666.
8. Morphew RM, Wright HA, LaCourse EJ, Woods DJ, Brophy PM. Comparative proteomics of excretory-secretory proteins released by the liver fluke *Fasciola hepatica* in sheep host bile and during in vitro culture ex host. *Mol. Cell. Proteomics*, 2007; 6: 963-972.
9. Tripathi T, Ghosh A, Todur VN, Kalita P, Vijayakumar R, Kalita J, Shukla R, Chetri PB, Shukla H, Sonkar A, Lyngdoh DL, Singh R, Chikara SK, Tripathi T. Draft genome of the liver fluke *Fasciola gigantica*. 2018; <https://doi.org/10.1101/451476>
10. Hillyer GV. *Fasciola* antigens as vaccines against fascioliasis and schistosomiasis. *Journal of Helminthology*, 2005; 79: 241-247.
11. Bennema S, Vercruyssen J, Claerebout E, Schnieder T, Strube C, Ducheyne E, Hendrickx G, Charlier J. The use of bulk-tank milk ELISAs to assess the spatial distribution of *Fasciola hepatica*, *Ostertagia ostertagi* and *Dictyocaulus viviparus* in dairy cattle in Flanders (Belgium). *Vet. Parasitol.* 2009; 165: 51-57.
12. Duscher R, Duscher G, Hofer J, Tichy A, Prosl H, Joachim A. *Fasciola hepatica* - Monitoring the Milky Way? The use of tank milk for liver fluke monitoring in dairy herds as base for treatment strategies. *Vet. Parasitol.* 2011; 178: 273-278.

13. Salimi-Bejestani MR, McGarry JW, Felstead S, Ortiz P, Akca A, Williams DJL. Development of an antibody-detection ELISA for *Fasciola hepatica* and its evaluation against a commercially available test. *Res. Vet. Sci.* 2005b; 78: 177-181.
14. Ozer B, Serin E, Gümürdülü Y, Gür G, Yilmaz U, Boyacıoğlu S. Endoscopic extraction of living *Fasciola hepatica*: "Case report and literature review". *Turk. J. Gastroenterol.* 2003; 14:74-77.
15. Cheung J, Enns R, Romney M, Reynolds S, Amar J. Biliary fascioliasis. *Gastrointestinal endoscopy.* 2005; 61(4):596-597.
16. Alatoon A, Cavuoti D, Southern P, Gander R. *Fasciola hepatica* Infection in the United States. *Labmedicine.* 2008; 39 (7): 425-428
17. Moll L, Gaasenbeek CP, Vellema P, Borgsteede FH. Resistance of *Fasciola hepatica* against triclabendazole in cattle and sheep in the Netherlands. *Veterinary Parasitology.* 2000; 91: 153-158.
18. Aksoy DY, Kerimoglu U, Oto A, Erguven S, Arslan S, Unal S, Batman F, Bayraktar Y. Infection with *Fasciola hepatica*. *Clin. Microbiol. Infect.* 2005; 11(11): 859-861.
19. Graham-Brown J, Williams LJD, Skuce PH, Zadoks NR, Dawes S, Swales H, Dijk VJ. Composite *Fasciola hepatica* faecal egg sedimentation test for cattle. *Veterinary record.* 2019; 10:1-8.
20. Coles GC. Anthelmintic resistance – looking to the future: a UK perspective. *Research in Veterinary Science.* 2005; 78: 99 -108
21. Kantzoura V, Kouam MK, Demiris N, Feidas H, Theodoropoulos G. Risk factors and geospatial modelling for the presence of *Fasciola hepatica* infection in sheep and goat farms in the Greek temperate Mediterranean environment. *Parasitology.* 2011; 138: 926-938.
22. Albishtue AA, Al-Alo KZ, A-Dujaily AH. Retrospective Survey of Liver flukes in Sheep and Cattle based on Abattoir Data in Al-Najaf Province, Iraq. *Acta Scientific Veterinary Sciences.* 2019; 1(4): 02-08.
23. Fuentes M, Malone J, Mas Coma S. Validation of a mapping and prediction model for human fasciolosis transmission in Andean very high altitude endemic areas using remote sensing data. *Acta. Trop.* 2001; 791: 87-95.
24. Zhang J, Si H, Zhou X, Shang X, Li B, Zhang J. High prevalence of fasciolosis and evaluation of the efficacy of anthelmintics against *Fasciola hepatica* in buffaloes in Guangxi, China. *Int. J. Parasitol. Parasites Wild.* 2019; 1 (8): 82-87.
25. Bhutto B, Arijo A, Phullan SM, Rind R. Prevalence of fascioliasis in buffaloes

- under different agro-climatic areas of Sindh Province of Pakistan. *International Journal of Agriculture and Biology*. 2012; 14: 241-245.
26. Khan MK, Sajid MS., Khan MN, Iqbal Z, Iqbal MU. Bovine fasciolosis: Prevalence, effects of treatment on productivity and cost benefit analysis in five districts of Punjab, Pakistan. *Research in Veterinary Science*. 2009; 87: 70-75.
27. Pfukenyi D, and Mukaratirwa S. Retrospective study of the prevalence and seasonal variation of *Fasciola gigantica* in cattle slaughtered in the major abattoirs of Zimbabwe between 1990 and 1999. *Onderstepoort Journal of Veterinary Research*. 2004; 71: 181-187.
28. Mungube EO, Bauni SM, Tenhagen BA, Wamae LW, Nginyi JM, Mugambi JM. The prevalence and economic significance of *Fasciola gigantica* and *Stilesia hepatica* in slaughtered animals in the semi-arid coastal Kenya. *Trop. Anim. Health Prod*. 2006; 38: 475-483.
29. Phiri AM. Common conditions leading to cattle carcasses and offal condemnations at 3 abattoirs in Western Province of Zambia and their zoonotic implications to consumers. *Journal of the South African Veterinary Association*. 2006; 77: 28-32.
30. Amer S, ElKhatam A, Zidan SH, Feng Y, Xiao L. Identity of *Fasciola* spp. in sheep in Egypt. *Parasites Vector*. 2016; 9: 623.
31. Kusumarini SR, Permata FS, Widyaputri T, Prasetyo D. Prevalence of fasciolosis emphasis on age, origin, body condition and post mortem by geographic information systems on sacrificial examination in Malang District – East Java. The 2nd International Conference on Computer Science and Engineering Technology, *Journal of Physics: Conference Series*. 2020; 1430 012025.
32. Byrne AW, McBride S, Lahuerta-Marin A, Guelbenzu M, McNair J, Skuce RA, McDowell SWJ. Liver fluke (*Fasciola hepatica*) infection in cattle in Northern Ireland: a large-scale epidemiological investigation utilising surveillance data. *Parasites & Vectors*. 2016; 9:209.
33. Fatmawati M, Herawati. Pada hewan kurban di kota batu Indonesian". *Journal of Halal*. 2017; 125-129.
34. Al-dujaily AH, Alatabi AC, Al-mialy AJ. Study the Rate of Hydatid Cysts, Liver Fluke, Pneumonia and Hepatitis in Al-Najaf Slaughter House, Al-Najaf, Iraq. *Kufa Journal for Veterinary Medical Sciences*. 2017; 8(2): 137-142.
35. Kadir AM, Rasheed AS. Prevalence of Some Parasitic Helminths among Slaughtered Ruminants in Kirkuk Slaughter House, Kirkuk, Iraq. *Iraqi*

- journal of veterinary Sciences*. 2008; 22(2): 81-85.
36. Bozorgomid A, Nazari N, Kia EB, Mohebbali M, Hajaran H, Heydarian P, Hamzavi, Nemati S, Aryaeipour M, Rokni MB. Epidemiology of fascioliasis in Kermanshah Province, western Iran. *Iran Journal of Public Health*. 2018; 47 (7): 967-972.
37. Khoramian H, Arbabi M, Osqoi M, Delavari H, Hooshyar and Asgari M. Prevalence of ruminant's fascioliasis and their economic effects in Kashan, center of Iran. *Asian Pac. J. Trop. Biomed*. 2014; 4(11): 918-922.
38. Arbabi M, Nezami E, Hooshyar H, Delavari M. Epidemiology and economic loss of fasciolosis and dicrocoeliosis in Arak, Iran. *Veterinary World*. 2018; 11(12): 1648-1655.
39. Murphy TM, Fahy KN, McAuliffe A, Forbes AB, Clegg TA, O'Brien DJ. A study of helminth parasites in culled cows from Ireland. *Prev. Vet. Med*. 2006; 76 (1-2): 1-10.
40. Ahmadi NA, Meshkehkar M. Prevalence and long term trend of liver fluke infections in sheep, goats and cattle slaughtered in Khuzestan, southwestern Iran. *J. Paramed. Sci*. 2010; 1:26-31.
41. Khosravi A, and Babaahmady, E. "Epidemiology of Fasciola hepatica in Iran". *Int. J. Biol*. 2012; 4(4): 86-90.
42. Moghaddam A, Massoud J, Mahmoodi M, Mahvi A, Periago M, Artigas P, Fuentes M, Bargues DM, Mas-Coma S. Human and animal fascioliasis in Mazandaran Province, northern Iran. *Parasitol. Res*. 2004; 94:61-69.
43. Theodoropoulos G. Risk factors and geospatial modelling for the presence of Fasciola hepatica infection in sheep and goat farms in the Greek temperate Mediterranean environment. *Parasitol*. 2011; 138:926-938.
44. Ansari-Lari M, Moazzeni M. A retrospective survey of liver fluke disease in livestock based on abattoir data in Shiraz, South of Iran. *Prev. Vet. Med*. 2006; 73: 93-96.
45. Sayadi M, Rezaei M, Jahanbakhsh MI, Gholamrezaei M, Mohammadpourfard I, Yahyaei M, Esmaeili, R. The prevalence of fascioliasis in slaughtered animals of the Industrial Slaughterhouse of Arak, Iran (2007-2010). *Iran. J. Health Sci*. 2015; 3(4): 59-64.
46. Mohammadpourfard I, Rezaei M, Sayadia M, Shariatifar N, Behzadi AA, Karimi F. Prevalence of dicrocoeliosis in slaughtered herbivores animals in Arak city of Markazi province in Iran. *J. Food Saf. Hyg*. 2015; 1(1): 18-21.
47. Alasaad S, Granados JE, Cano-Manual FJ, Meana A, Zhu XQ, Perez JM. Epidemiology of fasciolosis affecting Iberian ibex

- (Caprarilynaica) in southern Spain. *Parasitology Research*. 2008; 102:751-755.
48. Issia L, Pietrokovsky S, Sousa-Figueiredo J, Sttothard R, Wisnivesky-Colli C. Fasciola hepatica infections in livestock flock, guanacos and coypus in two wildlife reserves in Argentina. *Veterinary Parasitology* 2009; 165: 341-344.
49. Aminzare M, Hashemi M, Faz SY, Raeisi M, Hassanzadazar H. Prevalence of liver flukes infections and hydatidosis in slaughtered sheep and goats in Nishapour, Khorasan Razavi, Iran. *Veterinary World*. 2018; 11(2): 146-150.
50. Sardar A, Ehsan MA, Anower AKMM, Rahman MM, Islam MA. Incidence of Liver Flukes and Gastro-Intestinal Parasites in Cattle. *Bangl. J. Vet. Med*. 2006; 4 (1): 39-42.
51. Amarasinghe LD, Kumara HLNN. Effect of rainfall and temperature on liver and rumen fluke infestations of bovines in Sri Lanka. *Int. J. Biol. Chem. Sci*. 2007; 1(3): 229-236.
52. Barbosa R, Pinto C, Garcia P, Rodrigues A. Prevalence of fasciolosis in slaughtered dairy cattle from São Miguel Island, Azores, Portugal. *Vet. Parasitol. Reg. Stud. Reports*. 2019; 17: 100319.
53. Abdulhakim Y, Addis M. An Abattoir Study on the Prevalence of Fasciolosis in Cattle, Sheep and Goats in Debre Zeit Town, Ethiopia. *Global Veterinaria*. 2012; 8: 308-314.
54. Genet A, Derso S. Prevalence of Bovine Fasciolosis and Its Associated Risk Factor in and Around Dangila District, Awi Administration Zone, Northwestern Ethiopia". *European Journal of Biological Sciences*. 2015; 7: 114-119.
55. Assefa A, Assefa Z, Beyene D, Desissa F. Prevalence of bovine fasciolosis in and around Inchini town, West Showa Zone, Ada'a Bega Woreda, Central Ethiopia. *Journal of Veterinary Medicine and Animal Health*. 2015; Vol. 7(6): 241-248.
56. Mohammed C, Nigussie L, Dugasa J, Seid U. Prevalence of Bovine Fasciolosis and its Associated Risk Factors in Eastern Shoa, Kuyu District Central Ethiopia. *Arch. Vet. Sci. Technol*. 2018; AVST-140.
57. Affroze S, Begum N, Islam MS, Rony SA, Islam MA, Mondal MMH. Risk Factors and Gross Pathology of Bovine Liver Fluke Infection at Netrokona District, Bangladesh. *J. Anim. Sci. Adv*. 2013; 3(2): 83-90.
58. Gul N, Tak H, Fazilli KM, Abdullah I, Sofi AT. Prevalence of Fasciola infection in slaughtered Animals in Kashmir. *International Journal of Veterinary Sciences and Animal Husbandry*. 2016; 1(2): 30-36.
59. Tulu D, Gebeyehu S. Study of Prevalence and Associated Risk Factors of Bovine Fasciolosis in Jimma Horro District of Kellem Wollega Zone, Western Ethiopia. *Archives of Veterinary Science and Medicine*. 2018; 1: 009-018.