Clinically, Coprologically and Immunologically, *Fasciola hepatica* Detection in Wasit Province Buffaloes

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Abstract

The present search was performed to detect a predominance of Fasciola hepatica in buffaloes in Wasit Province, to investigate the related risk factors and to evaluate the efficacy of coprological (floatation and sedimentation) tests in comparison with serological indirect ELISA that considered as a gold standard technique in diagnosis of a disease. Out of 46 randomly selected buffaloes examined by fecal and serum blood samples, 6.5, 10.9, and 37%; were positives by floatation, sedimentation and ELISA tests, respectively. With ELISA, the degree of infection in positives was 70.9% mild, 24.9% moderate and no one had been strong. In dependence on the risk factors, the results of sex, age, body condition and feces consistency groups showed a greatly significant differences ($P>0.05$) in infection rates that recorded by the diagnostic techniques that used in this study. In related to serum total bilirubin, there was no significant differences between jaundiced and non-jaundiced groups ($P<0.05$).

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Keywords: Fasciolosis; Buffalo; Serology; Coprology; Iraq

التشخيص السريري والبرازي والمناعي لأصابة الجاموس بطفيلي (*Fasciola hepatica*) في محافظة واسط/ العراق

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الخلاصة

أجريت الدراسة الحالية لتحديد مدى انتشار المتورقة الكبدية في جاموس محافظة واسط ولتقصي عوامل الخطر المتعلقة بالإصابة ولتقييم فعالية اختبارات البراز (التطويف والترسيب) ومقارنتها مع اختبار الاليزا المصلي غير مباشر والذي اعتمد كتقنية اساسية في تشخيص المرض. من بين 46 راس من الجاموس، والتي تم اختيارها عشوائيا وفحصت بواسطة اخذ عينات من البراز ومن خلال فحص مصل الدم، وجد ان 6.5، 10.9، و37٪ على التوالي، كانت موجبة الاصابة بواسطة التطويف والترسيب والاليزا. من خلال اختبار الاليزا، كانت درجة الإصابة في الحيوانات موجبة الاصابة كالتالي 70.9٪ خفيفة و24.9٪ معتدلة ولم تمتلك أيا منها اصابة قوية. بالاعتماد على عوامل الخطر، أظهرت نتائج فحص المجموعات الخاصة بالجنس والعمر ووضع الجسم وطبيعة البراز، اختلافات احصائية كبيرة في معدلات الإصابة التي سجلت باستعمال تقنيات التشخيص المستخدمة في هذه الدراسة، $P>0.05$. وبما يتعلق بقياس نسبة البيليروبين الكلي في مصل الدم، لم يلاحظ وجود فروقات احصائية معنوية بين المجموعتين المصابة وغير المصابة باليرقان $P<0.05$.

الكلمات المفتاحية: داء المتورقات الكبدية، جاموس، فحص مصلي، فحص برازي، العراق.

Introduction

Fascioliasis or fasciolosis is a zoonoparasitic disease caused by plant-borne platyhelminthes (flatworm) trematodes of the genus *Fasciola* (1, 2, 3). Taxonomically, in spite of various species had recounted within *Fasciola*, just *F. hepatica* and *gigantica* had renowned to be a causes of fasciolosis (4). *Fasciola hepatica* infest a wide range of

animals like bovine, ovine, equine as well as deer, llamas, kangaroos, rabbits, beavers and rats, which demonstrating the extraordinary ability of the parasite for adapting to a newly hosts (5, 6, 7). *F. hepatica* has the widest prevalence in comparison with *F. gigantica*, and distributed about every clement areas where ruminants are emerged (8). Clinically, the effects of fasciolosis are well known and obvious when causes mortality; while sub-clinically, it mostly unnoticed and resulting in a marked economic losses such as the reducing in; nutrients performance, live-mass profit, milk yield and fertility, body quality and work output in draught animals, as well as a suppression in immunity and condemnation of livers during slaughter (9, 10, 11, 12). In certain diseased regions, the buffaloes at pasture, if compared with the restricted animals, had been with the same sensitivity to disease except that the hazard is lower (13). In farm animals, the confirmatory diagnosis of *F. hepatica* is usually done either by detection of the parasite's eggs or / and adults in feces and/or livers of infected animals by coprological and coproantigenic techniques, or by measuring of the specific antibodies against *F. hepatica* in blood, milk and meat juice with the serological techniques, and finally by the molecular technique. In first technique; that including the inspection, sedimentation and floatation; the procedure is simple and confirmatory but not useful as a diagnostic tool in low levels of adult fluke burdens and cannot be detect the infection at a pre-patent period because the eggs are found in feces when flukes are already matured usually between 10-14 weeks of infection (14, 15, 16, 17, 18, 19). While in other techniques, that involved an agar-gel precipitation test, agar gel diffusion test, indirect haemaglutination, counter electrophoresis, indirect immunofluorescent and ELISA, which developed to be as an alternative approaches to fecal eggs detection and/ or adult inspection, they have an ability to test even at low intensity infection, and large number of sera can be tested in a few time with detection of infection earlier than fecal eggs examination (20, 21, 22, 11, 23, 24). The diagnostic sensitivity and specificity of ELISA test that manufactured by SVANOVA company and performed by using the whole *F. hepatica* excretory- secretory antigens (ESAs), was (86-100)% and (83-97)%, respectively, according to (25, 26, 27, 28, 29, 30, 3, 31). The aims of study were: Identify a seroprevalence of *F. hepatica* in buffaloe, in Wasit Province/ Iraq, Evaluate the accurateness of the coprological methods (sedimentation and floatation) in compared with an indirect ELISA that used as a gold standard in detection of fascioliasis, Demonstrate the correlations between age, sex, body condition, consistency of feces and total bilirubin, with the infection.

Materials and Methods

- Area and animal (Study design): A total of 46 buffaloes were submitted to study during November and December / 2015, divided into two age groups, <4 years and > 4 years; and for three body's conditions statuses in depending on the body condition score (BCS); good BCS included the 4^{th} and 5^{th} scores, and a medium BCS involved the 3^{rd} score, while the poor buffaloes had the 1^{st} and 2^{nd} BCS that suggested by (32, 33). The study involved the animals that raised in various personal fields situated at Al-Hay district in Wasit province. In this region, the buffaloes grazed on natural pastures supplemented with cultivated pastures during the day. The random sampling was done to find out the clinic-seroprevalence of *F. hepatica* infection. The owners were involved in this study, just whom appeared their readiness for cooperativeness during the blood and data collection.
- Samples collection: The samples that obtained from each buffalo involved 5 ml blood from jugular vein and about 25 grams of fresh feces were obtained. The blood collected under aseptic condition by using a disposable syringe, installed in tubes without anticoagulant and transported to the diagnostic laboratory. A fresh fecal sample were collected directly from

rectum at early morning, placed separately in tightly closed universal bottles, kept with 10% formalin and then taken to the lab on same gathering's day (34).

- Diagnostic techniques :

- Coprological examination
  - Sedimentation: Two grams of feces in a conical cup were mixed with 20 ml of water and then leached through tea cleaner, then centrifuged for 3 minutes at 1500 rpm and by removing the supernatant, the sediment was added a drop of methylene blue and well mixed. A drop of sediment was taken into a slide to examine under microscope, eggs of *Fasciola* were identified by their characteristics yellow color (35, 36).
  - Floatation: "About 5 grams of feces was placed in a beaker, then 50 ml of Sodium chloride solution were poured into it and the content was mixed thoroughly using a stirring rod. The fecal suspension was strained by pouring it through a tea strainer into another beaker which was left to stand for 10 minutes and decanted. The bottom sediment was transferred into a test tube and resuspended with the floatation fluid to the brim. Finally, the test tube was covered with a cover slip and the cover slip mounted on microscopic slide for examination" (37, 38).
- Indirect ELISA: "SVANOVIR® *F. hepatica* - antibodies, is an indirect ELISA kit for detection of *F. hepatica* specific antibodies in bovine serum samples. In this procedure, samples are exposed to non-infectious *F. hepatica* antigen coated wells of microtitre plates. Antibodies (if present in the sample) bind to antigen in the wells. The HRP conjugate added subsequently to form a complex with antibodies. All reagents were equilibrated at room temperature 18-25°C before use and each strip labeled with a number. In duplicates, a 100µL of Positive Control Serum (reagent A) and 100µL of Negative Control Serum (reagent B) were added into selected wells, respectively, then the samples added with shaking a plate thoroughly. The plates sealed, incubated at 37°C for 1 hour and rinsed 4 times with PBS Tween Buffer solution. A 100µL of Conjugate was added to each well and incubated at 37°C for 1 hour and again rinsed 4 times with PBS Tween Buffer solution for removing the unbound material before the addition of substrate solution. Then, a 100µL of Substrate Solution was added to each well and incubated for 30 minutes in dark at room temperature. Subsequently, blue-green color was developed due to conversion of substrate by a conjugate. The reaction was stopped by adding a 50µL of Stop Solution to each well and mixed thoroughly. The optical density (OD) of the controls and samples was measured by using an automatic microplate reader (BioTek, USA) at a wavelength of 450nm. The optical density rate (ODR) values were calculated by using the following formula:-

$$ODR = OD_{\text{Sample or Control}} - OD_{\text{Negative Control}} / OD_{\text{Positive Control}} - OD_{\text{Negative Control}}$$

The manufacturer's current recommendations for interpretation of control and sample results test are detailed in (Table1)". Depending on antibodies' titration, the degree of infection divided into mild, moderate and strong if the results were 0.2-0.3, 0.3-0.4 and more than 0.4, respectively (29, 39, 40).

Table (1) Interpretation the results of *F. hepatica* ELISA kit (according to manufacturer)

Controls and Sample	ODR	Interpretation
$OD_{\text{Positive Control}}$	> 1	The results should fall within these values
$OD_{\text{Negative Control}}$	< 0.2	
Serum	< 0.4	No or low liver fluke burden
	≥ 0.4	Infection with liver fluke with likely production losses

- Total bilirubin: The method of action of the reagent total bilirubin, "kit is a diazo - sulfanilic acid method. The sulfanic acid reacts with sodium nitrite to form a diazotized sulfanilic acid. In the presence of dimethylsulfoxide, total bilirubin reacts

with the diazotized sulfanilic acid to form an azobilirubin. In absence of the dimethylsulfoxide, only the direct bilirubin will reacts to give azobilirubin" (41).

- Statistical Analysis: All data was labeled and analysed by a computerized IBM SPSS v.23 programme by using the descriptive analysis and Chi-square ($\chi 2$) to detect the recrudescence of infection and the correlations with the risk factors at a level $P<0.05$ (42).

Results

In table (2) that included the positive buffaloes with *F. hepatica* infection in Wasit province, from 46 animals and during two months (the period of the research), 3/46 (6.52%), 5/46 (10.9%) and 17/46 (37%) buffaloes were positive by floatation, sedimentation and indirect ELISA tests, respectively.

Table (2) The positive *F. hepatica* infections by the diagnostic techniques

Diagnostic Techniques		Positives		Negatives	
		No.	%	No.	%
Coprology	Floatation	3	6.52b	43	93.48
	Sedimentation	5	10.9b	41	89.1
Serology	Indirect ELISA	17	37a	29	63

The difference in small letters, vertically, referred to a significant differences at level P<0.05.

Table (3) The degree of infection in depending on the results of indirect ELISA test

Degree of infection	No.	%
Mildly positive	12	70.9a%
Moderately positive	5	29.4b%
Strongly positive	0	0c%

The difference in small letters, vertically, referred to a significant differences at level P<0.05.

In table (4) that deals with the relationships of risk factors with infection, the results were as follow:-

- In sex's group, the total positive states in 10/46 (21.7%) males, was 2/10 (20%); while in 36 females (78.3%), was 23/36 (63.89%).
- In age's group, and in less than 4 years, the total positives were 8/23 (34.8%); while in more than 4 years buffaloes, the total positives were 17/23 (73.9%).
- In the group of body condition, the total positives in good, medium and poor buffaloes were 2/8 (25%), 13/27 (48.1%) and 10/11 (90.9%), respectively.
- In feces consistency's group, 5/6 (83.3%), 19/38 (50%) and 1/2 (50%) had the diarrheic, normality and drying statuses, respectively.
- In the group of total bilirubin, the jaundiced buffaloes had 5/9 (55.6%) while the non-jaundiced had 20/37 (54.1%) positive cases.

Table (4) Prevalence of *F. hepatica* infection according to sex

Risk factors	Total buffaloes (n=46)	Positives			Total positives	Negatives
		Floatation	Sedimentation	ELISA		
Sex Male Female	10 (21.7%) 36 (78.3%)	0 (0%)b 3 (8.3%)a	1 (10%)b 4 (11.1%)b	1 (10%)b 16 (44.4%)a	2 (20%)b 23 (63.89%)a	8 (80%) 13 (36.1%)
Age <4 years >4years	23 (50%) 23 (50%)	1 (4.3%)b 2 (8.7%)a	1 (4.3%)b 4 (17.4%)a	6 (26.1%)b 11 (47.8%)a	8 (34.8%)b 17 (73.9%)a	15 (65.2%) 6(26.1%)
Body condition Good Medium Poor	8 (17.4%) 27 (58.7%) 11 (23.9%)	0 (0%)c 2 (7.4%)b 1 (9.1%)a	0 (0%)c 2 (7.4%)b 3 (27.3%)a	2 (25%)c 9 (33.3%)b 6 (54.5%)a	2 (25%)c 13 (48.1%)b 10 (90.9%)a	6 (75%) 14 (51.9%) 1 (9.1%)
Feces consistency Diarrhea Normal Dry	6 (13%) 38 (82.6%) 2 (4.4%)	1 (16.7%)a 2 (5.3%)b 0 (0%)c	1 (16.7%)a 4 (10.5%)b 0 (0%)c	3 (50%)a 13 (34.2%)b 1 (50%)ab	5 (83.3%)a 19 (50%)b 1 (50%)b	1 (16.7%) 19 (50%) 1 (50%)
Total bilirubin Jaundiced Non-jaundiced	9 (19.6%) 37 (80.4%)	1 (11.1%)b 2 (5.4%)b	1 (11.1%)b 4 (10.8%)b	3 (33.3%)b 14 (37.8%)b	5 (55.6%)b 20 (54.1%)b	4 (44.4%) 17 (45.9%)

The difference in small letters, vertically, referred to a significant differences at level P<0.05

Discussion

In Iraq, all studies related to buffalo's fascioliasis were, mostly, in depended on feces testing or inspection at slaughterhouse. In based on the indirect ELISA technique, the results were larger than these established by the coprological tests (floatation and sedimentation), and this could be related to the development of antibodies much earlier than presence of eggs in feces during the course of disease or because eggs ousted, intermittently, in dependant on an emptying of gall bladder (43, 44; 45, 46). Also, (47) demonstrated that the specific antibodies for excretory-secretory antigens of *F. hepatica* not detected in period that previously to infection, and these antibodies were reached at the peak after 3 weeks of infection. Also, he showed an absence of eggs or shed, intermittently, in feces during the acute and chronic stage, respectively. Therefore, the long pre-patent period of bovine fasciolosis render the coprological methods sensitive, only, at (8-9) weeks post infection, while the antibodies were detected at (1-5) week pre-fecal appearance of eggs, and supported the propose that until an animal infected with mature adults but might not excreted a detectable numbers of eggs in feces, and the ELISA technique was highly sensitive than coprology (25, 44, 45, 48). However, the levels of antibodies, in most animals, stay above the positive threshold of an indirect ELISA for about 12 weeks post treatment and this suggesting that the prevalence of data based on an ELISA may not be reliable about current infection status (49). In table (3), an absence of a strongly positives, with presence of a high rate of mildly positive in compartment with moderately infections, suggested that the theory that says" the buffaloes are more resistance to several infections than cattle and sheep, and this, may be attributed to the genetic resistance" (50, 51, 52, 53, 54). As well as, the present results observed a considerable increasing of infection rate in females as compared to males, especially by ELISA test. This fact might not interpreted accurately and probably presumed to that the male of buffaloes appear to be less adoptability to infection than female due a variation in production system, hormonal influences, shortness of male's life-spans or due to stress which causing suppression in immunity because of the females are grazed whereas males are confined (55, 56, 57). The age was seen as an important element in distribution of disease as observed in this study in buffaloes aged above 4 years that had more infection rate than from those less 4 years, and this could because either of long period of exposure to parasite in enclosed pasturing practice in flooded regions or for decreasing the immune-potency as a result of age's advance (58, 59, 60). The inequality in disease's prevalence between the different body conditions might be associated with the immunity and the needy feeding buffaloes that appeared to be less effectiveness for releasing from disease (61). (62) Reported that the average of food consumption decreased more than 15% after (20 weeks) of infection, and this may related to the reducing of buffalo's activity by decreasing of the essential substances that provided the growth and maintenance of life, or due to the substandard processes of dealing with or controlling things performed by buffalos' herder. In addition, the excess of weight gain in buffaloes could diminish fascioliasis may be related to the development of host's acquired immunity (63). The body condition was downfallen when these parasites begin in sucking of blood and tissue fluid with destruction a liver parenchyma because of migration the immature worms. So this is the most appropriate reason for those animals in order to loss their body condition in the case of chronic fasciolosis that consider as one of the commonest form of bovine disease that characterized by weight loss sign (64, 65). In relation to feces consistency, the diarrheic states were more prevalent in positives by a coprology than those by serology that had a normalcy for positives, and this result was in agreement with (66, 67). The results of total bilirubin test for examined animals (positives and negatives) don't show any

significant differences. During acute infection, the animal may had an anemia, weakness and jaundice which results from severe hemorrhage, which associated with infiltration of neutrophils and eosinophils, into peritoneal cavity as the larvae perforate a liver; presence an immature flukes in parenchyma, or due to trauma and feeding activity of adult flukes within bile ducts. However, during transition to a chronic phase and / or during therapies, the liver may heal and returned, relatively, to a normal status, refluxing on the total bilirubin's values to regain the normal levels (68, 69, 70, 71). In conclusion, this study demonstrated that the ELISA technique had the most reliable screening test for detection of *Fasciola hepatica* infection during the subacute, acute and chronic stages of disease.

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References

1. Pfukenyi, D. M.; Monrad, J. & Mukaratirwa, S. (2005). Epidemiology and control of trematode infections in cattle in Zimbabwe: a review: review article. J. S. Afr. Vet. Assoc., 76 (1): 9-17.
2. Mushtaq, S. (2011). An Epidemiological Study of Bovine Fasciolosis in Potohar region, Pakistan. Ph.D. Thesis, Department of zoology, Faculty of sciences, Arid Agriculture University, Rawalpindi. P. 2
3. Meerkhan, A. A.; Razak, A. H. & Younis, B. M. (2013). The differences between Direct examination and enzyme linked immunosorbent assay (ELISA) test, during the diagnosis of fasciolosis in Jaundiced slaughtered sheep in Duhok Abattoir, Kurdistan Region of Iraq. Int. J. Chem., Env. Biol. Sci., 1 (5): 707-709.
4. Mas-Coma, S.; Bargues, M. D. & Valero, M. A. (2005). Fascioliasis and other plant-borne trematode zoonoses. Int. J. Parasitol., 35 (11), 1255-1278.
5. Haseeb, A. N.; El-Shazly, A. M.; Arafa, M. A. & Morsy, A. T. (2002). A review on fascioliasis in Egypt. J. Egypt. Soc. Parasitol., 32 (1): 317-354.
6. Ashrafi, K.; Valero, M. A.; Panova, M.; Periago, M. V.; Massoud, J. & Mas-Coma, S. (2006). Phenotypic analysis of adults of Fasciola hepatica, Fasciola gigantica and intermediate forms from the endemic region of Gilan, Iran. Parasitol. Int., 55 (4): 249-260.
7. Fayez, M. S.; Alaa, A. M.; Adham, G. S.; Mohamed, K. Y.; Ali, O. E. & Ibraheem, M. G. (2015). Advanced and classical diagnosis of *Fasciola spp*. in Egypt. J. Am. Sci., 11 (5): 111-120.
8. Aleixo, M.; Freitas, D. F.; Dutra, L. H.; Malone, J.; Martins, I. V. & Molento, M. B. (2015). *Fasciola hepatica*: epidemiology, perspectives in the diagnostic and the use of geoprocessing systems for prevalence studies. Semina: Ciências Agrárias, 36 (3): 1451-1466.
9. Vassilev, G. D. & Jooste, R. (1991). Production losses and control of fasciolosis in cattle in Zimbabwe. Zimbabwe Vet. J., 22: 45-56.
10. Charlier, J.; Duchateau, L.; Claerebout, E.; Williams, D. & Vercruysse, J. (2007). Associations between anti-*Fasciola hepatica* antibody levels in bulk-tank milk samples and production parameters in dairy herds. Prev. Vet. Med., 78: 57-66.
11. Dorchies, P. (2007). Comparison of methods for the veterinary diagnosis of liver flukes (*Fasciola hepatica*) in cattle. Bulletin of University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca. Veterinary Medicine, 64 (1/2), 14-19.

12. Abunna, F.; Asfaw, L.; Megersa, B. & Regassa, A. (2010). Bovine fasciolosis: coprological, abattoir survey and its economic impact due to liver condemnation at Soddo municipal abattoir, Southern Ethiopia. Trop. Anim. Health Prod., 42 (2): 289-292.
13. Mezo, M.; Gonzalez-Warleta, M.; Castro-Hermida, J. A.; Muino, L. & Ubeira, F. M. (2011). Association between anti-*F. hepatica* antibody levels in milk and production losses in dairy cows. Vet. Parasitol., 180: 237-242.
14. Boray, J. C. (1985). Flukes of domestic animals, Parasites, pests and predators, World Animal Science Series, Elsevier, New York, Vol. B2, PP. 179-218.
15. Urquhart, G. M.; Armour, J.; Duncan, L.; Dunn, A. M. & Jennings, F. W. (1996). Veterinary Parasitology, 2nd ed., Blackwell Science, London, P. 110.
16. Duménigo, B. E.; Espino, A. M.; Finlay, C. M. & Mezo, M. (2000). Kinetics of antibodybased antigen detection in serum and faeces of sheep experimentally infected with *Fasciola hepatica*. Vet. Parasitol., 89 (1-2): 153-161.
17. Cucher, M. A.; Carnevale, S.; Prepelitchi, L.; Labbé, J. H. & Wisnivesky-Colli, C. (2006). PCR diagnosis of Fasciola hepatica in field-collected Lymnaea columella and Lymnaea viatrix snails. Vet. Parasitol., 137 (1): 74-82.
18. Charlier, J.; De Cat, A.; Forbes, A. & Vercruysse, J. (2009). Measurement of antibodies to gastrointestinal nematodes and liver fluke in meat juice of beef cattle and associations with carcass parameters. Vet. Parasitol., 166: 235-240.
19. Avcioglu, H.; Guven, E.; Balkaya, I.; Kaynar, O. & Hayirli, A. (2014). Evaluation of coprological and serological techniques for diagnosis of bovine Fasciolosis. Israel J. Vet. Med., 69 (4): 203-210.
20. Swarup, D.; Pachouri, S. P. & Sharma, B. (1987b). Comparative efficacy of sero-diagnostic tests in buffaloes naturally infected with *Fasciola gigantica*. Indian J. Anim. Sci., 57 (5): 373-376.
21. Cornelissen, J. B.; De Leeuw, W. A. & Van der Heijden, P. J. (1992). Comparison of an indirect haemagglutination assay and an ELISA for diagnosing Fasciola hepatica experimentally and naturally infected sheep. Vet. Q., 14 (4): 152-156.
22. Linh, B. K.; Thuy, D. T.; Saski, O. & Yoshihara, S. (2003). Application of agar gel diffusion test to the diagnosis of fasciolosis in cattle and buffaloes in the red river delta of Vietnam. Jpn Agric. Res. Q., 37 (3): 201-205.
23. Salam, M. M.; Maqbool, A.; Naureen, A. & Lateef, M. (2009). Comparison of different diagnostic techniques against Fasciolosis in Buffaloes. Vet. World, 2 (4): 129-132.
24. Dar, J. S.; Ganai, B. A.; Chishti, M. Z.; Shahardar, R. A.; Tantry, T. A.; Nizam, M. & Dar, S. A. (2014). Comparative analysis of different immunological techniques for diagnosing fasciolosis in sheep: A review. Biotechnol. Mol. Biol. Rev., 9 (3): 21-25.
25. Anderson, N.; Luong, T. T.; Vo, N. G.; Bui, K. L.; Smooker, P. M. & Spithill, T. W. (1999). The sensitivity and specificity of two methods for detecting Fasciola infections in cattle. Vet. Parasitol., 83 (1): 15-24.
26. Ortiz, P. L.; Claxton, J. R.; Clarkson, M. J.; Mcgarry, J. & Williams, D. J. (2000). The specificity of antibody responses in cattle naturally exposed to *Fasciola hepatica*. Vet. Parasitol., 93: 121-134.
27. Cornelissen, J. B.; Gaasenbeek, C. P.; Borgsteede, F. H.; Holland, W. G.; Harmsen, M. M. & Boersma, W. J. (2001). Early immunodiagnosis of fasciolosis in ruminants using recombinant *Fasciola hepatica* cathepsin L-like protease. Int. J. Parasitol., 31 (7): 728-737.

28. Salimi-Bejestani, M. R.; McGarry, J. W.; Felstead, S.; Ortiz, P.; Akca, A. & Williams, D. J. (2005). Development of an antibody-detection ELISA for *Fasciola hepatica* and its evaluation against a commercially available test. Res. Vet. Sci., 78 (2): 177-181.
29. Charlier, J.; De Meulemeester, L.; Claerebout, E.; Williams, D. & Vercruysse, J. (2008). Qualitative and quantitative evaluation of coprological and serological techniques for the diagnosis of fasciolosis in cattle. Vet. Parasitol., 153 (1): 44-51.
30. Kuerpick, B.; Conraths, F. J.; Staubach, C.; Froehlich, A.; Schnieder, T. & Strube, C. (2013). Seroprevalence and GIS-supported risk factor analysis of *Fasciola hepatica* infections in dairy herds in Germany. Parasitology, 140 (08): 1051-1060.
31. Van der Voort, M.; Charlier, J.; Lauwers, L.; Vercruysse, J.; Van Huylenbroeck, G. & Van Meensel, J. (2013). Conceptual framework for analysing farm-specific economic effects of helminth infections in ruminants and control strategies. Prev. Vet. Med., 109 (3): 228-235.
32. Ezenwa, V. O.; Jolles, A. E. & O'Brien, M. P. (2009). A reliable body condition scoring technique for estimating condition in African buffalo. Afr. J. Ecol., 47 (4): 476-481.
33. Alapati, A.; Kapa, S. R.; Jeepalyam, S.; Rangappa, S. M. & Yemireddy, K. R. (2010). Development of the body condition score system in Murrah buffaloes: validation through ultrasonic assessment of body fat reserves. J. Vet. Sci., 11 (1): 1-8.
34. Belić, B.; Cincović, M. R.; Stojanović, D.; Kovačević, Z.; Medić, S. & Simić, V. (2010). Hematology parameters and physical response to heat stress in dairy cows. Savremena poljoprivreda, 59 (1-2): 161-166.
35. Hanson, J. & Perry, B. (1994). The epidemiology, diagnosis and control of Helminth parasites of ruminants. A Hand Book, Food and Agricultural Organization of the United Nations, Rome, Italy, PP. 72-89.
36. Dorchies, P. (2008). Comparison of methods for the veterinary diagnosis of liver flukes (*F. hepatica*) in cattle. Bulletin of University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca. Vet. Med., 64 (1-2): 14-19.
37. Thienpont, D.; Rochette, F. & Vanparijs, O. F. J. (1979). Diagnosing Helminthiasis through coprological examination. Can. Vet. J., 22 (8): 247.
38. Edosomwan, E. U. & Chinweuba, C. R. (2012). A survey on helminth parasites of dogs in Benin city, Edo State, Nigeria. J. Vet. Med. Anim. Health, 4 (4): 56-60.
39. Sohair, I. B. & Eman, M. N. (2009). Histopathological and bacteriological studies on livers affected with fascioliasis in cattle. J. Comp. Path. Clin. Pathol., 22: 19- 45.
40. Claridge, J.; Diggle, P.; McCann, C. M.; Mulcahy, G.; Flynn, R.; McNair, J.; Strain, S.; Welsh, M.; Baylis, M. & Williams, D. J. (2012). *Fasciola hepatica* is associated with the failure to detect bovine TB in dairy cattle. Nature communications, 10.1038, 1840, 3, 853.
41. Riond, B.; Meli, M. L.; Braun, U.; Deplazes, P.; Joerger, K.; Thoma, R. & Hofmann-Lehmann, R. (2008). Concurrent infections with vector-borne pathogens associated with fatal anaemia in cattle: haematology and blood chemistry. Comp. Clinic. Path., 17 (3): 171-177.
42. Onwuegbuzie, A. J. & Leech, N. L. (2007). Sampling Designs in Qualitative Research: Making the Sampling Process More Public. Qualitative Report, 12 (2): 238-254.

43. Rodríguez-Osorio, M.; Rojas, J. & Gómez-García, V. (1998). *Fasciola hepatica*: Partial characterization of circulating antigens. J. Parasitol., 84 (5):1053-1055.
44. Reichel, M. P. (2002). Performance characteristics of an enzyme-linked immunosorbent assay for the detection of liver fluke (*Fasciola hepatica*) infection in sheep and cattle. Vet. Parasitol., 107 (1): 65-72.
45. Arias, M.; Hillyer, G. V.; Sanchez-Andrade, R.; Suarez, J. L.; Pedreira, J.; Lomba, C.; Diaz, P.; Morrondo, P.; Diez-Banos, P. & Paz-Silva, A. (2006). A 2.9 kDa *Fasciola hepatica*- recombinant protein based ELISA test for the detection of current-ovine fasciolosis trickle infected. Vet. Parasitol., 137 (1): 67-73.
46. Abebe, R.; Abunna, F.; Berhane, M.; Mekuria, S.; Megersa, B. & Regassa, A. (2010). Fasciolosis: Prevalence, financial losses due to liver condemnation and evaluation of a simple sedimentation diagnostic technique in cattle slaughtered at Hawassa Municipal abattoir, Southern Ethiopia. Ethiop. Vet. J.,14(1): 39-52.
47. Espino, A. M.; Marcet, R. & Finlay, C. M. (1997). *Fasciola hepatica*: Detection of antigenemia and coproantigens in experimentally infected rats. Exp. Parasitol., 85 (2): 117-120.
48. Aiello, S. E. & Mays, A. (2010). The Merck veterinary manual, 10th ed., Merck & Co Inc, New Jersey, PP. 1196-1198.
49. Castro, E.; Freyre, A. & Hernandez, Z. (2000). Serological responses of cattle after treatment and during natural re-infection with *Fasciola hepatica* as measured with a dot-ELISA system. Vet. Parasitol., 90 (3): 201-208.
50. Adams, L. G. & Templeton, J. W. (1998). Genetic resistance to bacterial diseases of animals. Rev. Sci. Tech. Off. Int. Epiz., 17 (1): 200-219.
51. Springbett, A. J.; MacKenzie, K.; Woolliams, J. A. & Bishop, S. C. (2003). The contribution of genetic diversity to the spread of infectious diseases in livestock populations. Genetics, 165 (3): 1465-1474.
52. Bishop, S. C.; Jackson, F.; Coop, R. L. & Stear, M. J. (2004). Genetic parameters for resistance to nematode infections in Texel lambs. Anim. Sci., 78 (2): 185-194.
53. Piedrafita, D.; Spithill, T. W.; Smith, R. E. & Raadsma, H. W. (2010). Improving animal and human health through understanding liver fluke immunology. Parasite Immunol., 32 (8): 572-581.
54. Hoffmann, I. (2010). Climate change and the characterization, breeding and conservation of animal genetic resources. Anim. Gen., 41 (s1): 32-46.
55. Molina, E. C.; Gonzaga, E. A. & Lumbao, L. A. (2005). Prevalence of infection with *Fasciola gigantica* and its relationship to carcase and liver weights, and fluke and egg counts in slaughter cattle and buffaloes in southern Mindanao, Philippines. Trop. Anim. Health Prod., 37 (3): 215-221.
56. Bhutto, B.; Arijo, A.; Phullan, M. S. & Rind, R. (2012). Prevalence of fascioliasis in buffaloes under different agroclimatic areas of Sindh Province of Pakistan. Int. J. Agric. Biol., 14 (2): 241-245.
57. Karim, M. R.; Mahmud, M. S. & Giasuddin, M. (2015). Epidemiological Study of Bovine Fasciolosis: Prevalence and Risk Factor Assessment at Shahjadpur Upazila of Bangladesh. Immunol. Infec. Dis., 3 (3): 25-29.
58. Sánchez-Andrade, R.; Paz-Silva, A.; Suárez, J. L.; Panadero, R.; Pedreira, J.; López, C.; Díez-Baños, P. & Morrondo, P. (2002). Influence of age and breed on natural bovine fasciolosis in an endemic area (Galicia, NW Spain). Vet. Res. Comm., 26 (5): 361-370.

59. Phiri, A. M.; Phiri, I. K.; Sikasunge, C. S. and Monrad, J. (2005). Prevalence of fasciolosis in Zambian cattle observed at selected abattoirs with emphasis on age, sex and origin. J. Vet. Med., Series B, 52 (9): 414-416.
60. Yildirim, A.; Ica, A.; Duzlu, O. & Inci, A. (2007). Prevalence and risk factors associated with Fasciola hepatica in cattle from Kayseri province, Turkey. Revue de médecine vétérinaire, 158 (12): 613.
61. Nuraddis, I.; Pawlos, W. & Tadele, T. (2010). Prevalence of bovine fasciolosis and economic importance due to liver condemnation at Kombolcha industrial abattoir, Ethiopia. Int. J. Vet. Med., 8 (2): 1937-8165.
62. Ferre, I.; Barrio, J. P.; Gonzalez-Gallego, J. & Rojo-Vazquez, F. A. (1994). Appetite depression in sheep experimentally infected with *Fasciola hepatica*. Vet. Parasitol., 55 (1): 71-79.
63. Chanie, M. & Begashaw, S. (2012). Assessment of the economic impact and prevalence of ovine fasciolosis in Menz Lalo Midir District, Northeast Ethiopia. Vet. World, 5 (5): 261-264.
64. Radostits, O. M.; Gay, C. C.; Hinchcliff, K. W. & Constable, P. D. (2007). Veterinary medicine. A textbook of the diseases of cattle, horses, sheep, pigs and goats. 10th edition, W.B. Saunders Company Ltd, London, PP. 1576-1579.
65. Ozung, P. O.; Owai, P. U. & Oni, K. O. (2011). An Assessment of the Prevalence of Fascioliasis of Ruminants in Ikom Abattoir of Cross River State, Nigeria. Continental J. Vet. Sci., 5 (1): 1-5.
66. Sripa, B.; Kaewkes, S.; Intapan, P. M.; Maleewong, W. & Brindley, P. J. (2010). Food-borne trematodiases in Southeast Asia: epidemiology, pathology, clinical manifestation and control. Adv. Parasitol., 72: 305-350.
67. Adrien, M. D. L.; Schild, A. L.; Marcolongo-Pereira, C.; Fiss, L.; Ruas, J. L.; Grecco, F. B. & Raffi, M. B. (2013). Acute fasciolosis in cattle in southern Brazil. Pesquisa Veterinária Brasileira, 33 (6): 705-709.
68. Kaneko, J. J.; Harvey, J. W. & Bruss, M. L. (2008). Clinical biochemistry of domestic animals. Academic press, PP. 212-218.
69. Arslan, F.; Batirel, A.; Samasti, M.; Tabak, F.; Mert, A. & Ozer, S. (2012). Fascioliasis: 3 cases with three different clinical presentations. Turk. J. Gastroenterol, 23 (3): 267-271.
70. Haçarız, O.; Sayers, G. & Baykal, A. T. (2012). A proteomic approach to investigate the distribution and abundance of surface and internal *Fasciola hepatica* proteins during the chronic stage of natural liver fluke infection in cattle. J. Proteome Res., 11 (7): 3592-3604.
71. Gokce, H. I.; Gokce, G. & Cihan, M. (2007). Alterations in coagulation profiles and biochemical and haematological parameters in cattle with traumatic reticuloperitonitis. Vet. Res. Comm., 31 (5): 529-537.