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ULTRASONORGRAPHIC FEATURES OF THE LIVER AND GALL BLADDER OF WATER BUFFALOES WITH PATENT *Fasciola spp.* INFECTION

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ABSTRACT

Seventy-nine purebred and crossbred water buffaloes consisting of 43 animals without fasciolosis and 36 animals positive for fasciolosis through fecalysis were utilized to determine the ultrasonographic features of the liver with patent fasciolosis. Sedimentation technique of fecal samples revealed mixed parasitic infection of *Fasciola* spp. with strongyles, *Moniezia* sp., and *Eimeria* spp. However, besides *Fasciola* spp., the other parasites are not known to cause liver damage. Ultrasonographic features observed in the water buffaloes with patent *Fasciola* spp. infection were significantly more echogenic liver parenchyma due to presence of multiple hyperechogenic foci, thicker hyperechoic gall bladder wall, wider gall bladder lumen and less echoic gall bladder lumen. However, other abnormal liver and gall bladder conditions should be ruled out. The thickness of the liver parenchyma was not different between the two groups. The results of the study suggest that ultrasonography can be used as a complementary tool for diagnosis of patent fasciolosis in water buffaloes.

Key words: *Fasciola*, fasciolosis, gall bladder, liver, ultrasonography

INTRODUCTION

In the Philippines, dairy water buffalo is one of the major sources of milk products today (Constante and Acorda, 2012). However, buffalo raising in the Philippines is faced with many obstacles. Among these are parasitisms, particularly fasciolosis. Fasciolosis continues to be the most economically important parasitic disease of domestic ruminants in the country as shown by early and recent studies (Arañez, 1962; Tongson, 1978; Domingo, 2013; Gordon *et al.*, 2015).

Fasciolosis is caused by liver flukes *Fasciola* spp. that utilize freshwater lymnaeid snails as intermediate hosts. This contributes to fasciolosis-associated risks in water buffaloes because of the natural behavior of these animals in finding aquatic areas to wallow leading to exposure to the infective stage of the parasites. Fasciolosis occurs as a subclinical infestation most of the time but there are gradual or progressive production losses. According to Copeman and Copland (2008), the major economic importance can be seen in the reduced milk yield, fertility and meat harvest. Moreover, the major concern of our local farmers would be the additional costs that would come from the anthelmintic treatments and milk-withdrawal periods after the treatment.

Diagnosis of fasciolosis in water buffaloes is a challenge presented to the veterinarians more than the local dairy farmers. It is a difficult task to eradicate the disease and the agent itself so whenever possible, an early diagnosis could really help in the prompt treatment of fasciolosis to minimize damage to infected animals and contamination of the environment with eggs.

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There are various ways to determine the presence of *Fasciola* spp. infection (Alvarez Rojas *et al.,* 2014). The classical method of coproscopy (fecal examination) is inexpensive but it is relatively not highly sensitive and only applicable to patent infections. Serological methods for fecal antigens or serum antibodies detection have higher sensitivities but require performance of the tests in diagnostic laboratories. In human fasciolosis, ultrasonography is becoming a popular way of diagnosis in other countries (Richter *et al.*, 1999; Kanoksil *et al.*, 2006; Taheri *et al.*, 2007; Gandhi *et al.*, 2010; Alizadeh *et al.*, 2011; Sakru *et al.*, 2011; Abdi *et al.*, 2013; Karadag-Oncel *et al.*, 2012).

Very few studies have used ultrasonography in water buffaloes to assess the features of an abnormal organ. This study aimed to investigate if ultrasonography could be used in diagnosing fasciolosis in dairy water buffaloes by describing ultrasonograms of the liver and gall bladder of water buffaloes without fasciolosis and water buffaloes with patent *Fasciola* spp. infection. In spite of its cost, a positive result will provide comparative advantage of this technique over the other methods because it can be used for on-site diagnosis even if only one animal is involved.

MATERIALS AND METHODS

A total of 79 one to five years old Bulgarian Murrah, Italian Murrah and crossbred water buffaloes were utilized in the study. Of this number of animals, 43 were without fasciolosis (normal group) based on history and previous regular fecal examination. The animals were housed in the Philippine Carabao Center National Headquarters and Genepool in Muñoz, Nueva Ecija. The rest of the animals (36) were with patent *Fasciola* spp. infection based on finding of *Fasciola* eggs upon fecal examination. These were from different smallhold buffalo dairy farms with history of fasciolosis in Magdalena, Laguna and Pililla, Rizal. Animals with patent *Fasciola* spp. infection exhibited weight loss, decreased milk production and diarrhea.

For fecal examination, fecal samples were collected *per rectum* and each sample (at least 50 g) was placed in a labeled Ziploc® plastic bag. Samples were transported in an ice chest to the Veterinary Parasitology Laboratory of the Department of Veterinary Paraclinical Sciences, College of Veterinary Medicine, University of the Philippines Los Baños for microscopic fecal examination (FE) using the sedimentation procedure as described by Zajac and Conboy (2012) with modifications. Briefly, approximately 10 g of fecal sample was placed in a narrow-bottomed container with 100 ml water. The mixture was mixed thoroughly using an electric drill with a drill bit for mixing attachment. The suspension was passed through a metal sieve (~200 µm mesh size) and the filtrate collected into another narrow-bottomed cup. This was allowed to stand for 15 min and decanted. Water was added and the sedimentation-decantation steps were repeated. A drop of the solution was placed in a slide, covered with a coverslip and examined microscopically using 10x objective. *Fasciola* eggs were identified based on morphology (Soulsby, 1982). Other parasite forms were also recorded.

Ultrasonographic examination was performed in a non-fasted standing, non-sedated animal using an ultrasound machine (HS-2200V, Honda Electronics Co., Ltd., Aichi,

Japan) equipped with a 2.0 MHz multifrequency linear array scanner (HLV-4214M, Honda Electronics Co., Ltd., Aichi, Japan). Ultrasound gel (Trans gel®, Rothmeier Laboratories, Inc., Philippines) was applied to the transducer and the skin of the animal prior to examination to avoid air interposition.

To facilitate liver examination, a rectangular area on the right side of the animal bounded dorsally by the spinous processes, ventrally by the right paramedian area, cranially by the shoulder and caudally by the flank was shaved to ensure that the examination area was free from hair that might interfere with the results. The scanner was placed on the dorsal to middle aspect of the 9th-12th intercostal spaces (ICS) to visualize the liver, and the middle aspect of the 9th-11th ICS for the gall bladder. Examination was done during maximum inspiration of the animal. During the examination the ultrasound features of the structures of the liver and gall bladder were noted.

The ultrasonograms of the two organs were examined and different ultrasound features were determined. The echogenicity of the organ structures were evaluated through digital analysis using Adobe Photoshop CC (Adobe Photoshop, Inc., USA. 2015) and echo mean values were obtained. Measurement data were presented as Mean±SD in centimeters. Means and SD were computed in Microsoft Excel 2007. Ultrasonographic findings were compared between the two groups of animals using two-tailed t-test using OpenEpi v.3 (www.openepi.com/Menu/OE_Menu.htm).

The protocol used in the study was approved by the Institutional Animal Care and Use Committee of the College of Veterinary Medicine, University of the Philippines Los Baños.

RESULTS AND DISCUSSION

Fecal examination using modified sedimentation technique revealed that some of the water buffaloes have mixed parasitic infestations. Although flotation methods are commonly used in detecting ova in domestic animals, sedimentation methods or combined sedimentation and flotation are mostly used for trematode visualization because of relatively higher density of their eggs (Johansen *et al.*, 2010; Alvarez Rojas *et al.*, 2014).

Besides *Fasciola* spp. eggs, strongyle eggs followed by *Moniezia* sp. eggs and *Eimeria* spp. oocysts were commonly found in fecal samples of the water buffaloes. *Moniezia* sp. eggs were least observed among the fecal samples. Eggs of these tapeworms may be found in fecal samples but diagnosis is usually by finding proglottids (Soulsby, 1982).

The present findings are in agreement with several studies, (Mamun *et al.*, 2011; Rinaldi *et al.*, 2009; Kobak and Pilarczyk, 2012), wherein the highest prevalence of gastrointestinal parasite in water buffaloes would either be *Fasciola* sp. or strongyles. However, strongyles, *Moniezia* and *Eimeria* coccidia in water buffaloes are not known to cause liver damage.

The liver parenchyma from the group without fasciolosis showed uniform hypoechoic parenchyma which is even throughout the organ (Fig. 1) whereas the buffaloes with patent *Fasciola* spp. infection (Fig. 2) generally showed a heterogeneous hepatic parenchyma with diffused multifocal hyperechogenicity. Based on t-test, the

mean echo values showed that it was significantly much higher (p=0.00) in the water buffaloes with patent fasciolosis (101.1) than those without fasciolosis (73.7) (Table 1). The hyperechogenicities scattered in the parenchyma might be due to the presence of adult flukes in smaller bile ducts, focal areas of cellular infiltration, fibrotic lesions or calcification due to previous migrations of flukes or immature flukes that were still migrating. Hard calcified mass in the different parts of liver parenchyma were among the gross lesions observed in *F. gigantica*-infected cattle (Affroze *et al.*, 2013). In buffaloes with chronic fasciolosis, Tharwat (2012) stated that presence of numerous echogenic foci in the liver parenchyma is a common imaging finding. However, it is also possible that it could be due to abnormal liver conditions such as fatty infiltration or hepatic tumors. These could be verified by histopathological examination of biopsy samples.

The echo mean values of the liver parenchyma obtained in both groups in this study were greater than the value reported by Acorda and Alejandro (2007) in non-lactating water buffaloes. The difference might be attributed to the thickness of the subcutaneous fat in the abdominal wall in purebred buffaloes compared to the crossbred animals used in other studies. Echo mean values of the liver parenchyma drop in hydropic degeneration and rise during fatty infiltration (Constante and Acorda, 2012) which could also be the reason behind the increase in values because these findings cannot be stated as a pathognomonic finding during fasciolosis.

The gall bladder was not visualized in all the animals sampled with ultrasonography

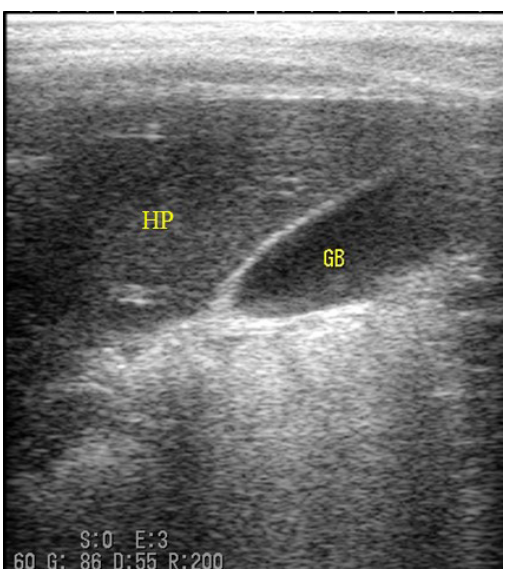

Fig. 1. Ultrasonogram of the liver and gall bladder in a water buffalo without fasciolosis. The hepatic parenchyma (HP) is evenly hypoechoic throughout the organ. The gall bladder (GB) showed a slightly anechoic lumen with echogenic walls.

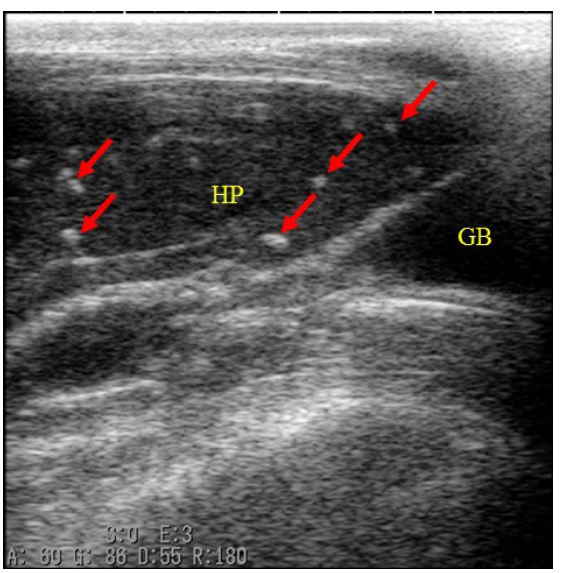

Fig. 2. Ultrasonogram of the liver and gall bladder in a water buffalo with patent *Fasciola* spp. infection. There is presence of heterogeneous hepatic parenchyma (HP) with diffused multifocal hyperechogenicity (red arrows). The gall bladder (GB) also appears dilated with anechoic lumen with a hyperechoic wall.

Table 1. Echo mean values (Means±S.E.) of the liver parenchyma and gall bladder lumen of apparently normal water buffaloes without fasciolosis and with patent *Fasciola* spp. Infection.

Structure	Without fasciolosis	With patent *Fasciola* spp. infection	Probability value
Liver parenchyma	73.7±1.49[a] (n=43)	101.1±1.63[b] (n=36)	0.00
Gall bladder lumen	34.2±2.15[a] (n=27)	27.2±2.43[b] (n=22)	0.01

n=number of observed samples
In a row, values with different superscripts were significantly different (P<0.05).

because the rumen would sometimes block the view of the organ during inspiration. The gall bladder of water buffaloes without fasciolosis had smooth and hyperechoic walls with an anechoic lumen (Fig. 1), which is in agreement with some of the observed ultrasonograms in other studies (Acorda and Alejandro, 2007). The gall bladder of the water buffaloes with patent *Fasciola* spp. infection showed anechoic lumen with a thickened hyperechoic wall (Fig. 2), which is also in agreement with some previous studies in sheep (Gonzalo-Orden *et al.*, 2003) and cattle and buffaloes (Tharwat, 2012). Based on analysis, the gall bladder wall of the animals with *Fasciola* infection was significantly thicker (p=0.01) than those without fasciolosis (Table 2). This could be due to the effect of flukes in the bile ducts. Thickening of the bile duct wall is among the prominent pathological changes observed in fasciolosis (Molina *et al.*, 2008; McGavin and Zachary, 2006). Although this is usually observed in bile ducts containing the flukes (Soulsby, 1982), it is possible that it may extend to the gall bladder wall due to dissemination of products of the parasite that stimulate inflammation, hyperplasia and fibrosis. In the study of Tharwat (2012), the gall bladder wall of *Fasciola* spp. infected cattle appeared thickened and edematous. In thickened gall bladder wall with no accompanying signs of cholestasis, it could be due to edema rather than inflammation (Braun, 2009). In this study, the mean diameter of the gall bladder lumen in the *Fasciola*-infected group was also significantly higher (p=0.05) than those of the buffaloes without fasciolosis (Table 2). This may indicate that the gall bladder of the infected animals was distended, which could be attributed to the fluke infection. Ferre *et al.* (1995) observed a decrease in bile flow in experimentally infected sheep with *F. hepatica* 6-14 weeks after infection, which concides with the pre-patent and patent periods of infection. However, gall bladder distention may also be caused by a decreased food intake resulting in lack of stimulation of the reflex emptying process (Tharwat, 2012).

In relation to the mean echo values of the gall bladder lumen, it was significantly much higher (p=0.01) in buffaloes without fasciolosis (Table 1). This may be attributed to the normal bile production of the water buffaloes without fasciolosis whereas a subsequent decrease in the gall bladder lumen echogenicity would indicate that there was lesser production of the bile components due to hepatic damage caused by the flukes. In experimental *F. hepatica* infection in sheep, bile flow as well decreased bile acid and biliary bilirubin secretion have been observed (Ferre *et al.,* 1995).

Table 2. Mean±S.E. thickness (cm) of the liver parenchyma, gall bladder wall and gall bladder lumen diameter of apparently normal water buffaloes without fasciolosis and with patent *Fasciola* spp. infection.

Organ/Structure	Without fasciolosis	With patent *Fasciola* spp. infection	Probability value
Liver parenchyma	8.2±0.12[a] (n=43)	8.2±0.13[a] (n=36)	0.86
Gall bladder wall	0.4±0.01[a] (n=32)	0.5±0.02[b] (n=22)	0.01
Gall bladder lumen	3.5±0.18[a] (n=32)	4.1±0.21[b] (n=22)	0.05

The mean thickness of the liver parenchyma had no significant difference between the two groups (Table 2). Affroze *et al.* (2013) observed at necropsy that *F. gigantica*-infected cattle appear to have larger liver. Although it was not mentioned, it is possible that the increase in size could be in terms of the width of the infected livers rather than their thickness. This may explain the absence of difference in the mean thickness of the liver of the two groups of water buffaloes in this study. In comparison to the ultrasonographic measurements obtained by Acorda and Alejandro (2007) in non-lactating water buffaloes, greater liver thickness values were obtained in this study. The source of variation could be due to difference in the physiological status of some of the animals used in this study, which were lactating and were purebred water buffaloes. Purebreds are known to be heavier and bigger than crossbred buffaloes (Constante and Acorda, 2012).

Finally, in infected buffaloes where the bile duct was imaged, mineralization as showed by intense echoes accompanied by distal acoustic shadowing that was observed by Tharwat (2012) in some chronically infected cattle and water buffaloes was not seen in this study.

The present results suggest that ultrasound features such as echo mean values of the liver and gall bladder lumen, gall bladder lumen width and gall bladder wall thickness of water buffaloes with *Fasciola* spp. can be distinguished from water buffaloes without fasciolosis. Thus, for faster diagnosis of patent fasciolosis, ultrasonography along with clinical history may be considered in place of fecal examination.

Future studies using ultrasonography to demonstrate moving adult *Fasciola* in the liver of infected animals and to characterize other parasitic infections of water buffaloes are recommended. Monitoring the ultrasonographic features of the liver of water buffaloes during the pre-patency to patency periods should be done to determine the usefulness of this technique for early diagnosis of fasciolosis.

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