Case Report

OVASCOPIC AND HEPATOSONOGRAPHIC FINDINGS IN A COW WITH CONCURRENT INFECTION OF PARAMPHISTOME AND *Fasciola gigantica* FROM CAUVERY DELTA REGION OF TAMIL NADU

M. Venkatesan[1], A. Latchumikanthan[2], M. Saravanan[3], M. Veeraselvam[1], K. Jayalakshmi[1], M.K. Vijayasarathi[2], A. Meenakshisundaram[4], S. Yogeshpriya[1] and N. Premalatha[5]

Department of Veterinary Medicine
Veterinary College and Research Institute
Tamil Nadu Veterinary and Animal Sciences University
Orathanadu, Tamil Nadu, India

ABSTRACT

A six years old Jersey crossbred cow was presented to Veterinary Clinical Complex, Veterinary College and Research Institute, Orathanadu, Thanjavur district with the history of jowl edema and voiding foul smelling diarrhoea for a month. On clinical examination of the cow, pale conjunctival mucous membrane, jowl edema, moist muzzle and watery malodorous diarrhoea, body temperature 38.4 0C, heart rate - 64 bpm, respiratory rate - 28 bpm were found. Ultrasonography of liver parenchyma showed hyperechoic small areas of hepatic parenchyma with dilated hepatic vein, portal vein and caudal vena-cava and gall bladder showed distension with presence of hyperechoic sediments. On cholecystocentesis under ultrasound guidance with 20 Gauge 1.5" needle at right costochondral junction at 9th intercostal space, collected bile sample revealed Fasciola gigantica eggs. Faecal examination revealed eggs of both Paramphistomes and Fasciola gigantica. Measurement of eggs of parasites was carried by micrometry which confirmed 151 x 72 µm size Amphistome eggs in feces and 162 x 91 µm size Fasciola gigantica eggs in both bile and fecal samples. The cow was treated with oxyclozanide @ 18mg/kg body weight orally and supportive therapy. Good improvement was noticed in the health status of the animal after treatment. Further examination of faecal samples from that animal did not show any parasite eggs on 15th, 30th and 45th

Corresponding author, email: drvenksmvsc88@gmail.com
[1]Assistant Professor
[2]Assistant Professor, Department of Veterinary Parasitology
[3]Assistant Professor, Veterinary Clinical Complex
[4]Professor and Head Department of Veterinary Parasitology
[5]Professor and Head

day after treatment. In this study, ultrasonography of liver and gall bladder combined with faecal examination was helpful in detection of concurrent paramphistomosis and fasciolosis. The present case seems to be the first report of mixed infection of Fasciola sp and paramphistome in cattle from the Cauvery Delta region of Tamil Nadu.

Key words: Cattle, Cauvery delta, *Fasciola gigantica*, Liver, Paramphistomosis, Ultrasound.

Case history and observation

Six-year-old Jersey cross bred cow (250 kg) in second month of lactation was presented to Veterinary Clinical Complex, Veterinary College and Research Institute, Orathanadu with a history of jowl edema and voiding malodorous diarrhoea for a period of one month. Physical examination revealed pale conjunctival mucous membrane, jowl edema, moist muzzle and malodorous diarrhoea. The values for vital parameters were temperature 38.4^{0}C, heart rate- 64 bpm, respiratory rate - 28 bpm. Ruminal consistency was doughy and contraction was 3/5min. Whole blood was collected in EDTA vial for hematology and clotting tubes for serum biochemistry. Faecal samples collected were subjected for parasitological examination.

Ultrasonographic examination

Ultrasonography of the thorax and abdomen was done using (Esaote My lab version 1) 2.5 to 5 MHz curvi-linear probe as per the previous reports (Braun, 2009). Heart, pericardium, reticulum, spleen, thoracic examination by ultrasound on left side from 4th to 8th intercostal space was performed. On right side abdomen kidney, intestine, omasum (Braun, 1990), liver and gall bladder (Braun, et al 2005) were examined from 9th to 12th intercostal space. Cholecystocentesis was performed under ultrasound guidance with 20 Gauge 1.5" needle at right side costochondral junction in the 10th intercostal space as per previous report (Braun, et al 1995) (Fig. 1a, b). Around 10 ml of bile collected and subjected for physical, parasitological examinations.

Faecal examination

Faecal sample was processed by both sedimentation and floatation methods and examined for parasitic eggs. One gram of fecal sample was processed for detection of egg per gram of feces (EPG) by following the Stoll's dilution method (Soulsby, 1982).

Animal grazing area was traced in Pattukotai, Thanjavur district. Grazing pattern of the present cattle was free range roaming. Authors found snails in and around the water body near cattle grazing areas (Fig.4a-d). The collected snails were identified as per the standard keys given by Rao (1989).

Treatment and discussion

The animal was treated with Inj. Ringers lactate @ 10ml per kg 2.5 L IV, Inj. Vit B_1 B_6 B_{12} plus Liver extract 10ml IM, Inj. Flunixin @1 mg/kg IM, kaolin powder 100g orally for 3 days. Suspension Oxyclozanide @ 18.7 mg/kg body weight orally (Zanil® Virbac Pharmaceuticals Pvt. Ltd) was given two doses at 48 hours interval (Radostits *et al.,* 2010), Liver tonic (Liv - 52®Himalaya Animal Health) 50 ml per day was administered every day for a period of one month. On initial day of presentation, the animal had microcytic

hypochromic anaemia, elevated ALT and AST (Table.1).

Ultrasonography of liver parenchyma showed presence of hyperechoic small areas of hepatic parenchyma with dilated hepatic vein, portal vein and caudal venacava. At 10th intercostal space the gall bladder showed distension with presence of hyperechoic sediments, suggestive of bile sediments or eggs of helminth parasites (Fig. 2a, b).

Ultrasound guided cholecystocentesis revealed dark transparent greenish bile with whitish sediments observed. Bile sample showed greenish colour in appearance, pH - 8.0, total protein 6 g/ dl and specific gravity of 1.035 by refractrometer. Upon centrifugation of bile sample at 2500 rpm for 5 minutes, collected sediments were examined under low power of light microscope. Microscopic examination of bile revealed eggs of Fasciola with yellowish tint and measuring162 x 91 µm size (Fig. 3c).

Fig. 1 (a & b). Ultrasound guided cholecystocentesis with 20 Gauge 1.5" needle at right side costochondral junction at the 10th intercostal space.

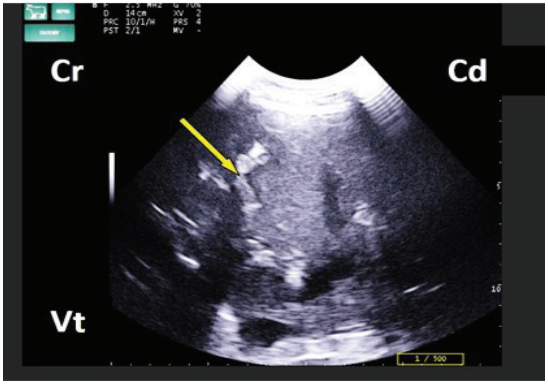

Fig. 2a. Ultrasonography of liver at 12th intercostal space shows hyperechoic small areas of hepatic parenchyma (arrow) with dilated hepatic vein

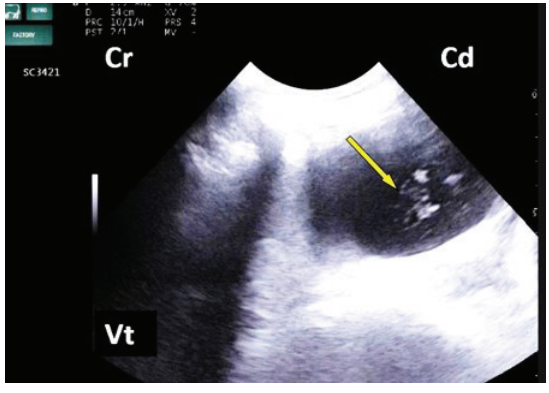

Fig. 2b. Ultrasonography of gall bladder (anechoic) at 10th intercostal space shows hyperechoic internal echos (arrow).

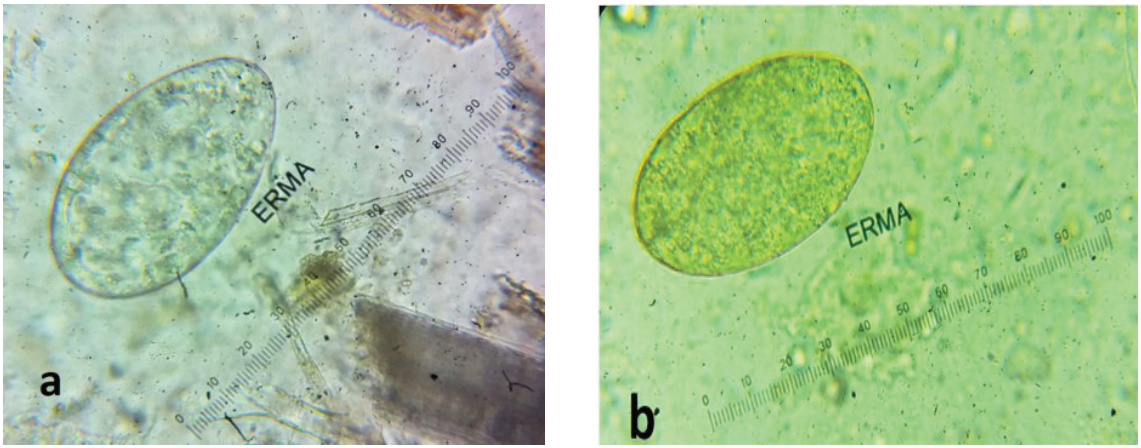

Fig. 3 (a & b). Faecal examination showed Amphistome eggs measuring 151 x 57µm, yellowish *Fasciola gigantica* eggs measuring 162 x 91µm (x400)

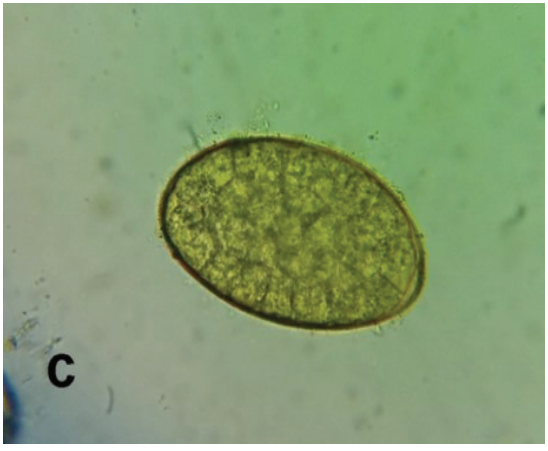

Fig. 3c. Bile examination showed yellowish *Fasciola gigantica* eggs measuring 162 x 91µm (x400)

Fecal sample examination by sedimentation method revealed presence of both amphistome eggs and yellowish *Fasciola gigantica* eggs. Measurement of eggs of parasites was carried using the micrometry which confirmed 151 x 72 µm and 162 x 91 µm size eggs of amphistome and *Fasciola gigantica*, respectively (Fig. 3a, b). Floatation method of faecal examination did not reveal any eggs. Egg per gram of faeces (EPG) of 400 and 600 was observed for *Fasciola gigantica* and Paramphistomes, respectively in quantitative faecal examination.

Good improvement was noticed in the health status of the animal after treatment. Further examination of faecal samples from that animal did not show any parasite eggs on 15th and 30th day after treatment. Animal owner was advised to give deworming for the cattle in every 3 months.

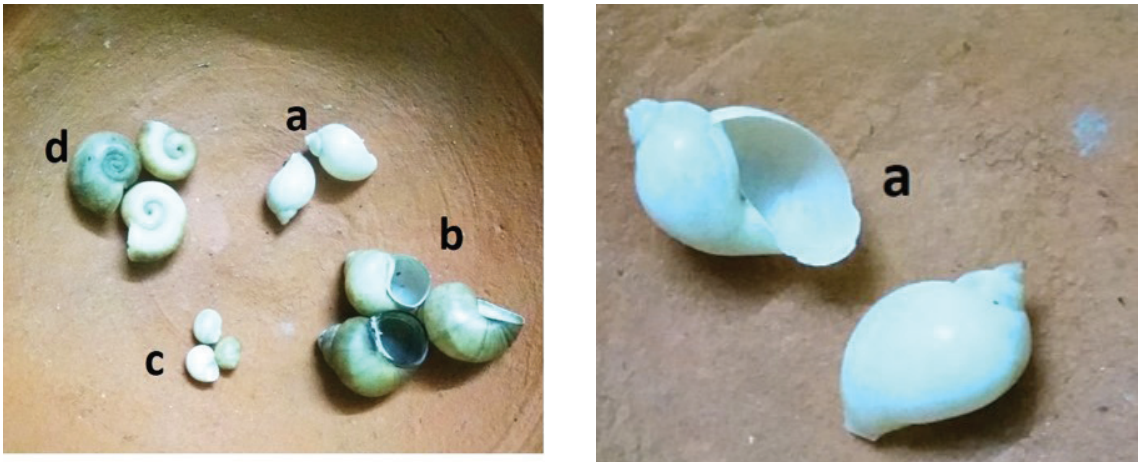

Fig. 4. Snails collected from animal grazing areas. (a) *Lymnaea* sp., (b) *Bellamyia* sp., (c) *Stenothyra* sp., (d) *Indoplanorbis* sp.

Several reports were published in cattle with mixed infection of trematodes around the world. Concurrent infections with different trematodes species are quite common, because they share snail as an intermediate host and similar transmission site (Keyyu *et al.,* 2006; Phiri *et al.,* 2006). Yabe *et al.* (2008) reported that there is no significant cross-protection in cattle infected with amphistome, *F. gigantica* and Schistosoma in endemic areas of Zambia, Africa.

Gastro-intestinal parasites cause considerable economic loss to the marginal and small-scale farmers. Among the gastrointestinal parasites affecting cattle and buffaloes, flukes play a major role to cause production loss and mortality in India (Juyal *et al.,* 2003). These flukes include *Fasciola hepatica*, *Fasciola gigantica* and paramphistomes affecting the large ruminants. Fasciolosis is the most economically important helminthic infection of ruminants in Asia and Africa caused by *Fasciola gigantica* (Spithill *et al.,* 1997). Bovine fasciolosis was reported previously from various parts of India as 4.76% in cattle and 7.81% in buffaloes from Tamil Nadu by Latchumikanthan and Soundararajan (2013), 10.79 % in cattle and 13.09% in buffaloes from Pantnagar by Garg *et al.* (2009). Acute fasciolosis and in prepatent cases detection by faecal examination is not possible and also it takes time. Gupta and Yadav (1992) reported that faecal examination technique does not allow the detection of early stage prepatent infection in fasciolosis, which lasts approximately 13 weeks. El-Damaty *et al.* (2018) reported that ultrasound is a valuable prognostic technique for assessment of bovine fasciolosis.

Table 1. Haematology and Serum biochemistry of cow with concurrent Paramphistomes and *Fasciola gigantica* infection

Parameters	Before Treatment	After Treatment (60th day)	Reference value (Radostits *et al.,* 2010)
Hb (g/dl)	6.8	8.7	8-15
PCV (%)	47	24.05	24-46
RBC (mill/cmm)	3.4	6.18	5-10
WBC (/cmm)	8757	9845	4,000-12,000
Neutrophils (%)	42	37.9	15-45
Lymphocytes (%)	55	49.8	45-75
Monocytes (%)	2	2.4	2-7
Eosinophils (%)	1	8.5	2-20
Basophils (%)	0	1.5	0-2
MCV (fl)	13.82	39	40-60
MCH (pg)	2	14.1	11-17
MCHC (g/dl)	14.46	36.1	30-36
Platelet 10^3 / µl	511	525	1,00,000 – 8,00,000
Total protein (g/dl)	6.9	8.4	5.7-8.1
Albumin (g/dl)	2.60	2.34	2.1-3.6
Globulin (g/dl)	3.70	6.06	3.0-3.5*
Albumin: globulin ratio	0.70	38.61	0.84-0.94*
Glucose (mg/dl)	47	67	45-75
AST (U/L)	72	54	45-110
ALP (U/L)	767	325	0-500
ALT (U/L)	56	22	30
BUN (mg/dl)	22	23.3	7.8-24.6
Creatinine (mg/dl)	1.81	0.75	0.6-1.8
Calcium (mg/dl)	9.8	10.8	9-12
Phosphorus (mg/dl)	6.44	6.54	5.5-6.5
Fibrinogen (g/dl)	9.0	4.0	2.0-7.0
Plasma protein: Fibrinogen	7.2	19.5	10 - 37*

In the present case there were focal areas of hyperechoic hepatic parenchyma and gall bladder distension with hyperechoic sediments and could be due to presence of adult flukes in liver and Fasciola eggs in bile, respectively. This concurs with previous studies of Braun *et al.* (1995) Field investigation on animal rearing areas identified the *Lymnaea sp., Indoplanorbis sp., Bellamyia sp.* and *Stenothyra sp.* of snails (Fig. 4), in which Lymnaea sp. Snails were main source of spread of infective stages of trematodes on the plantations nearby water source. Similarly, Sundararajan *et al.* (2018) identified Radix luteola, Indoplanorbis exustus, Gyraulus convexiusculus, Melanoides tuberculatus, Bellamya crassi spiralis, Vivipara, Stenothyra blanfordiana snails from North Eastern zone (Kancheepuram and Vellore) and hilly zone (The Nilgiris) of Tamil Nadu. Soundararajan *et al.* (2000) reported that Lymnaeid snails act as intermediate host for *Fasciola sp.* and Biswas (1991) revealed that Indoplanorbis exustus snails were responsible for spreading diseases paramphistomiasis and schistosomiasis in domestic animals.

Shokier *et al.* (2013) reported oxyclozanide, oxyclozanide and levamizole combination and triclabendazole were found having high efficacy against fasciolosis with 100% reduction in faecal egg count in the day 14 post-treatment. This was in agreement with the present study.

Clinical, faecal, bile and ultrasonographic examination of the liver and gall bladder in this cow revealed the actual cause of the enteritis due to concurrent infection of *Fasciola gigantica* and Paramphistomes.

ACKNOWLEDGEMENT

Authors are thankful to the Dean, Veterinary College and Research Institute, Orathanadu and Director of Clinics, TANUVAS for providing the necessary facilities.

REFERENCES

Biswas, G. (1991). Snail-borne diseases and their role in veterinary public health. In: Snails, Flukes and Man. Zoological Survey of India, Calcutta. pp. 73-78.

Braun, U. (1990). Ultrasonographic examination of the liver in cows. *American Journal of Veterinary Research,* **513**: 1522-1526.

Braun, U. (2009). Ultrasonography of the gastrointestinal tract in cattle. *Veterinary Clinics of North America: Food Animal Practice*, **25** (3):567-590.

Braun, U., Pospischil, A., Pusterla, N. and Winder, C. (1995). Ultrasonographic findings in cows with cholestasis. *Veterinary Record*, **137**: 537–543.

Braun, U., Schweizer, G. and Pospischil, A. (2005). Clinical and ultrasonographic findings in three cows with rupture gall bladders. *Veterinary Record*, **156**: 351-353.

El-Damaty, H.M., Mahmmod, Y.S., Gouda, S.M., and Sobhy, N.M. (2018). Epidemiological and ultrasonographic investigation of bovine fascioliasis in smallholder production system in Eastern Nile Delta of Egypt. *Preventive Veterinary Medicine*, **158**: 35-42.

Garg, R., Yadav, C.L., Kumar, R.R., Banarjee, Vastsya, S. and Godara, R. (2009). The epidemiology of fasciolosis in ruminants in different geo-climatic regions of north India. *Tropical Animal Health and Production*, **41**:1695–1700.

Gupta, S.C. and Yadav, S.C. (1992). Sexual maturity of *Fasciola gigantica* in experimentally infected rabbits, goats and buffaloes. *Indian Journal of Parasitology*, **16**:133–134.

Juyal, P.D., Kasur, K., Hassan, S.S. and Paramjit, K. (2003). Epidemiological status of paramphistomiasis in domestic ruminants in Punjab. *Journal of Parasitology Diseases*, 231-235.

Keyyu, J.D., Kassuku, A.A., Msaliwa, L.P., Monrad, J. and Kyvsgaard, N.C. (2006). Cross-sectional prevalence of helminth infections in cattle of traditional, small scale and large-scale dairy farms in Iringa district, Tanzania. *Veterinary Research Communications,* **30**: 45–55.

Latchumikanthan, A. and Soundararajan, C. (2013). Serodiagnosis of bovine fasciolosis by enzyme linked immuno sorbent assay in Chennai, Tamil Nadu. *Indian Journal of Animal Sciences,* **83** (6): 587–590.

Phiri, A.M., Phiri, I.K. and Monrad, J. (2006). Prevalence of amphistomiasis and its association with *Fasciola gigantica* infections in Zambian cattle from communal grazing areas. *Journal of Helminthology*, **80**:65–68.

Radostits, O.M., Gay, C.C., Hinchcliff, K.W. and Constable, P.D. (2010). Veterinary Medicine. A text book of the diseases of cattle, sheep, goats, pigs and horses. 10th edition. Elsevier Publication.

Rao, N.V.S (1989). Handbook Freshwater Molluscs of India. Zoological Survey of India, Calcutta. The Radiant Process Pvt Ltd., Calcutta. pp. 289.

Shokier, K.M., Aboelhadid, S.M. and Waleed, M.A. (2013). Efficacy of five anthelmintics against a natural *Fasciola* species infection in cattle. *Journal of Basic and Applied Sciences*, **2**:41-45.

Soulsby, E.J.L. (1982). Helminths, arthropods and protozoa of domesticated animals. 8th ed. English Language Book Society and Baillere Tindal, London.

Soundararajan ,C., Anil Kumar, R. and Iyue, M. (2000). Prevalence of fasciolosis in sheep in Nilgiris. *Indian Journal of Animal Research,* **34**:73-74

Soundararajan, C., Venkatesan, R. and Nagarajan, K. (2018). Prevalence of snails in north eastern and hilly zones of Tamil Nadu, India. *International Journal of Zoology Studies,* **3**(3):29-32.

Spithill, T.W., Piedrafita, D. and Smooker, M. (1997). Immunological approaches for the control of fasciolosis. *International Journal of Parasitology*, **27**: 1221-1235.

Yabe, J., Phiri, I.K., Phiri, A.M., Chembensofu, M., Dorny, P. and Vercruysse, J. (2008). Concurrent infections of *Fasciola*, *Schistosoma* and *Amphistomum spp.* in cattle from Kafue and Zambezi River basins of Zambia. *Journal of Helminthology*, **82:** 373–376.