## Journal of Advanced Veterinary Research Volume 3 (2013) 83-85



**Short Communications** 

## Hepatic Pseudo-Fasciola Infection in German Shepherd Dogs

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Accepted 18 February 2013

Fascioliasis is a common parasitic disease of small and large ruminants and other ungulates. In dogs, the disease usually occurred by feeding of raw liver infected with immature liver flukes (Dalton, 1998). Pseudo-Fascioliasis refers to the presence of Fasciola eggs in the stool resulting not from an actual infection but from recent ingestion of infected livers containing eggs. This situation can be avoided by having the patient follow a liver-free diet several days before a repeated stool examination (Espino and Finlay, 1994; Demerdash et al., 2011). Extrahepatic fascioliasis has been reported in the subcutaneous tissue (Aguirre et al., 1981), brain (Ruggieri et al., 1967), lungs (Couraud et al., 1975), epididymis (Aguirre et al., 1981), inguinal lymph nodes (Arjona et al., 1995) and in gastrointestinal system organs (Acosta-Ferreira et al., 1979) and the cecum (Park et al., 1984). Pseudo-Fasciolosis increases the percentages of false positive diagnosis that depends on detection of Fasciola antigen in cattle faeces (Anderson et al., 1999). In this study, we investigated the problem of Pseudo-Fascioliasis in German Shepherd dogs by stool analysis, estimation of serum enzymes, bilirubin and ultrasonography.

In this study, four German shepherd puppies of three months old and their dam were admitted to the Teaching Hospital of Medicine and Infectious Diseases (Department of Medicine and Infectious Diseases at the Faculty of Veterinary, Medicine, Cairo University) for routine vaccination. They exposed to the routine clinical examination including stool analysis. They were kept as inpatient for 5 days and they were fed on liver free diets. The case histories were recorded focusing on type of food and previous medication. The owner fed their dogs on raw bovine liver slices and he brought some liver slices for examination.

Stools were collected from the four puppies and dam daily for analysis. Blood-sera were collected and tested for serum enzymes (alanine aminotransferase (ALT) and aspartate aminotransferase (AST) and bilirubin. The liver slices were examined to detect Fasciola worms and their eggs.

The collected stool samples were examined by concentration sedimentation techniques (Denham and Suswillo, 1995). The Fasciola eggs were counted daily (Happich and Boray, 1969). To differentiate between pseudo and true Fasciola infections, in pseudo-infection, numbers of eggs per gram stool get down and eggs completely disappear while in true infection, numbers of eggs per gram stool remain within stable range.

Serum enzymes (ALT and AST) and bilirubin (total and direct) were estimated using commercial kits supplied by Spinreact Company, Spain, and according to Sodikoff (2001).

The dogs were examined by ultrasonography using the ultrasound device (Toshiba, Japan) at Department of Surgery, Anesthesiology and Radiology. Ultrasonography aimed to confirm if the Fasciola infection is pseudo or true. The liver tissues, gall bladders and bile ducts were examined to detect any abnormal changes.

The liver slices were examined for detection of whole Fasciola flukes or their heads that probably hidden inside the bile ducts. The slices were also

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washed with tap water and the collected water was centrifuged, then the sediment was microscopically examined to detect Fasciola egg.

We found that the golden yellow oval operculated eggs measuring up to 170  $\mu$ m long by 80 $\mu$ m wide, that were identified as Fasciola eggs (Dalton, 1998; Stephen and Richard, 2001; Aitken, 2007). The number of Fasciola eggs per gram stool declined daily as shown in Table 1.

Ultrasonography showed normal liver tissue architecture, common bile ducts, bile ductules and gall bladder as illustrated in Figures 1 and 2



Fig.1. Ultrasonographic image showed normal liver architecture and gall bladder of German shepherd dog.

Serum levels of ALT, AST, total and direct bilirubin in the examined dogs were within the normal range as presented in Table1.

Examination of the liver slices revealed presence of leaf like flukes in bile ductules; it measures up to 7.5 cm long by approximately 1.2 cm wide. Characteristically, it has less well-defined shoulders and long straight sides that were identified as *Fasciola gigantica* worms (Stephen and Richard, 2001; Aitken, 2007). The microscopical examination of the sediment of the collected water used to wash liver slices showed presence of Fasciola eggs.



Fig. 2. Ultrasonographic image showed normal liver, bile duct and ductules of German shepherd dog.

True Fascioliasis, the flukes mature after 3 - 4months post- infection begins to layout eggs. Fasciola fluke secretes an average of 9000-25000 eggs/day. Fasciola induce remarkable harmful hepatic pathological alterations and it has a number of immune evasion mechanisms that allow for chronic fascioliasis to occurred (Stephen and Richard, 2001). In Pseudo-Fascioliasis, nothing of the previous harmful changes was occurred. This may verified by the followings (i) Stool analysis and stool egg count for the examined dogs showed that the Fasciola egg count were declined until disappeared at the 4th day from the beginning of stool analysis and stopping feeding on liver. (ii) Liver damage or bile duct obstruction were excluded by estimation the levels of ALT, AST, total bilirubin and direct bilirubin in sera of the examined dogs. Aitken (2007) reported that ALT, AST, total and direct bilirubin levels can indicate chronic disease once Fasciola adult flukes are present in the biliary tree and may provide useful prognostic indicators. (iii) Normal architecture of liver and the patency

Table1. Stool analysis, serum enzymes, bilirubin and ultrasonography of liver of examined dogs

	Age (month)	Sex	Stool analysis (number of Fasciola/EPG)					Serum enzymes		Bilirubin		Ultrasonography of liver
			1st Day	2nd Day	3rd Day	4th Day	5th Day	ALT	AST	Т	D	of liver
Puppy-1	3	F	7	2	0	0	0	37	29	0.5	0.1	Normal
Puppy-2	3	F	9	1	0	0	0	42	45	0.4	0.06	Normal
Puppy-3	3	M	5	3	2	0	0	56	38	0.45	0.07	Normal
Puppy-4	3	F	4	2	0	0	0	29	13	0.15	0.11	Normal
Dam	24	F	11	4	1	0	0	60	54	0.55	0.17	Normal

Normal values (Kaneko et al., 1997): Bilirubin: T: Total (0.1-0.5 mg/dl), D: Direct (0.06-0.12 mg/dl), Serum enzymes: ALT (21-102 IU/L), AST (23-66 IU/L)

of gall bladder and bile ducts were also confirmed by ultrasonography. Moreover, in Pseudo-Fascioliasis, the Fasciola eggs are passed through the digestive tract of the infected dogs without any change because it is highly resistant to the digestive enzymes and secretions (Dalton, 1998).

In conclusion, Pseudo-Fascioliasis could occur in dogs eating liver infected with Fasciola flukes and misdiagnosed during the stool analysis of dogs. It could be differentiated true-Fascioliasis and Pseudo-Fascioliasis by stopping feeding on liver, with daily stool analysis at least for 5 days. Measuring serum enzymes and bilirubin and finally ultrasonography of liver, gall bladder and bile duct may employ as a confirmatory aiding procedures.

## Acknowledgment

Authors are pleased to thank the Department of Medicine and Infectious Diseases and Department of Surgery, Anaesthesiology and Radiology, Faculty of Veterinary Medicine, Cairo University, for providing the laboratories and devices during this research work.

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