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PREVALENCE AND PATHOLOGY OF *DICTYOCAULUS VIVIPAROUS* INFECTION IN CATTLE AND BUFFALOES

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ABSTRACT

The nematode, *Dictyocaulus viviparus*, causes severe parasitic bronchitis in dairy animals and is responsible for significant economic losses. Present study was conducted to determine the occurrence of bovine lungworm *Dictyocaulus viviparus* a highly endemic parasite. The fecal samples were collected from buffaloes (n=235) and cattle (n=210) and examined using Baermann technique. After slaughtering the lungs of all the buffaloes and cattle were separated and carefully examined for the presence of *D. viviparus* at Faisalabad abattoir. Overall non-significant difference in prevalence of *D. viviparus* in fecal samples of cattle (4.76%) and buffaloes (5.10%) was recorded. Grossly morbid lung tissues from buffalo (n=5) and cattle (n=3) revealed nodular appearance, severe pneumonia and congestive changes. Multifocal lymphocytic and eosinophilic bronchiolitis along with widened interlobular pulmonary septa were observed in lungs sections infected with *D. viviparus.* Lungs tissue exhibited peribronchiolar aggregation of mononuclear cells, presence of eggs and different developmental stages including, newly hatched and L1 stage of *D. viviparus* in air spaces.

Key words: Buffaloes, Cattle, Nematode, *Dictyocaulus viviparus*, Lungs, Pathology.

INTRODUCTION

Livestock is an important sector in Pakistan's economy and contributes about 11.9% in the GDP of Pakistan. This sector is considered to be a net source of invariable income for rural and middle grade agri-business holders and contributes about 55.4% in year 2012-13 in the agriculture sector as compared to the 55.3% in year 2011-12 (Anonymous, 2012-2013). Livestock farming in Pakistan is rapidly progressing and it contributes significantly in national economy (Babar *et al*., 2012; Hussain *et al.,* 2012; Abubakar *et al*., 2012). Dairy industry suffers from different diseases and among them parasitic problem is important in hindering the development of dairy sector (Woolley, 1997; Alim *et al*., 2012).

In livestock rearing, in some complex conditions/situations like decreased milk production due to unknown reason, increased treatment cost in any disease, increased labor charges and different parasitic diseases culling of animals become necessary (Khan *et al*., 2013). The control of various parasitic diseases is paramount importance to decrease the incidence of production disorders. Several parasitic infectious agents are responsible for respiratory diseases in livestock population. However, dictyocaulosis is considered to be a potentially increasing and costly problem (Ploeger, 2002), responsible for verminous pneumonia (Larsson *et al.,* 2011; Laabs *et al.,* 2012; Zaman *et al*., 2012). The lungworm (*Dictyocaulus viviparus*) is a relatively common parasite in tropical and subtropical areas and causes heavy economic losses (Panciera and Confer, 2010). The parasite causes a severe pulmonary disease in cattle commonly called as parasitic bronchitis, dictyocaulosis, or husk (Taylor *et al.,* 2007). Affected herds usually indicate high disease prevalence and mortality depending on the degree of pasture contamination (Cantacessi *et al.,* 2011). Clinical signs in naturally affected animals are: loss of appetite, reduced growth, increase respiratory rate and coughing (Verstegen *et al.,* 1989). *Dictyocaulus viviparus* has been implicated as a parasite that causes high mortality in cattle (Panuska, 2006). Healthy animals get infection through intake of contaminated grass. Chronic inflammatory changes in infected lungs were in the form of ciliated epithelial cells loss, peribronchiolitis, eosinophilic bronchiolitis, and atelectasis (Nashiruddullah *et al.,* 2007). In Pakistan, nematodes infections in sheep and goats have been reported (Ahmed *et al.,* 2006) however; scanty information is available about the naturally occurring cases of *D. viviparus* infection and its pathology in indigenous cattle and buffaloes. Therefore, the current study was designed to reveal the prevalence and frequency of gross and histopathological lesions due to *Dictyocaulus* infection in slaughtered dairy cattle and buffaloes.

MATERIALS AND METHODS

Study area: Pakistan is situated in South Asia with tropical and subtropical weather conditions. Faisalabad, the second largest city of Punjab and third largest city of

Pakistan, is located at latitude 31° - 26' N, longitude 73° – 06' E and altitude 184.4m. It has long summer episode that extends from May to September. In summer, ambient temperature ranges from 30 to 45°C and even sometimes may go to 48°C. A severe hot and humid (humidity 45.38±13.11%) period begins from mid-July, extends up to mid September (Khan *et al.,* 2011; Farooq *et al*., 2013).

Management of animals: In Pakistan, mainly buffaloes and cattle are kept in small clusters (<10 animals) in rural areas. Milch animals are usually stall-fed with wheat straw, green fodder and concentrates (mostly cottonseed cake); however, young animals are grazed in newly harvested fields, on canal banks, roadsides and wastelands. Stall feeding and grazing of animals is frequently carried out throughout the year.

A total of 235 buffaloes and 210 cattle brought to the Faisalabad abattoir, Pakistan were screened for the presence of *D. viviparus* in their feces. Male (n=140) and female (n=95) buffaloes were divided into three age groups, i.e., >1 year, <1-3 years and above three years. Similarly, all the cattle (n=210) were also divided into male (n=125) and female (n=85) and into three age groups.

Fecal Examination: About 25 g of fresh fecal sample was collected from rectum of each animal prior to slaughtering. The collected samples were brought to laboratory and stored at room temperature. The eggs of *D. viviparus* were recovered using Baermann's technique and examined under microscope (Shahzad *et al*., 2012).

Gross and histopathological study: After slaughtering all the animals were examined for the presence of any lesions on lungs. Out of these animals, five buffaloes and three cattle showed pneumonic changes. The trachea, bronchi and bronchioles of these animals were explored carefully for the presence of the adult lungworms. Affected lung tissues were preserved in 10% neutral buffered formalin and processed by the routine method of dehydration and paraffin embedding. 4-5µm thick tissue sections were finally stained with hematoxylin and eosin and examined under microscope (Sikandar *et al.,* 2012). Microscopic lesions were scored following the method already described (Jung *et al*., 2012).

Data analysis: The data collected in present study were analyzed by using Chi-square test. Odd ratio and 95% CI were determined and $P<0.05$ was considered as significance level.

RESULTS AND DISCUSSION

In the present study, overall (4.88%) prevalence of *D. viviparus* was recorded, while the prevalence of this nematode in cattle and buffaloes was 4.76% and 5.10%, respectively, however; difference between these species was non-significant. Overall the prevalence of dictyocaulosis was higher in young animals in both species (Table 1). Previously, higher prevalence (8-34%) of *D. viviparus* in different dairy herds and in sheep has been reported in various tropical, subtropical and temperate regions of different countries (Alasaad *et al.,* 2009; Bennema *et al.,* 2010; Addis *et al.,* 2011). The heavy rain fall during hot and humid period favor the survival of infective nematode larvae in lush green pastures which may support the development of these nematode and more chances of ingestion by buffaloes and cattle. Though *D. viviparus* infection had been reported from tropical and sub-tropical countries, like Brazil (Silva *et al.,* 2005), India (Sharma and Dhar, 1987), Malaysia (Lat-Lat *et al.,* 2010) and Turkey (Yildiz, 2006) but countries having temperate climate are not spared from this parasite (Ireland - Murphy *et al.,* 2006; Germany - Schnieder *et al.,* 1993; Netherlands - Ploeger *et al.,* 2000; Sweden - Hoglund *et al.,* 2004). *D. viviparus* lung worm infection has been identified in wild life such as roe deer (Divina *et al.,* 2000) and wild cervids (Pybus, 1990).

In the present study, prevalence of *D. viviparus* was non-significantly ($P>0.210$) higher in young animals (Table 1). It could be due to the reason that the older animals may develop immunity and did not shed larvae of nematodes (Holzhauer *et al*., 2011; Strube, 2012). Grossly, lungs of animals exhibited nodular appearance, congestion, pleural adhesion and purulent exudation in cattle and buffaloes (Table 2). Numerous adult cylindrical worms were abundantly present in the dorso-caudal bronchi and inside the terminal branches of caudal bronchioles. Lungs were consolidated and frothy material was also observed in bronchi.

Histologically massive exudate was present in the bronchioles predominantly comprises of eosinophils, lymphocytes, macrophages and giant cells. Degenerative and necrotic changes were observed in bronchiolar epithelium. Some histological sections exhibited catarrhal bronchitis and atelectatic changes in association with pronounced eosinophilic granulomatous alveolitis. Increased numbers of goblet cells and the peribronchiolar lymphoid tissue were observed. Cluster of eggs of *D. viviparous*, new hatched larvae in alveoli and ruptured interalveolar septae were observed in lungs of both cattle and buffaloes (Fig. 1). Alveolar septa were thick due to cellular infiltration, slight fibroplasia and inconsistent proliferation of type II pneumocytes (Fig. 2).

Different developmental stages including newly hatched *D. viviparus* larvae have also been reported in ruminants (Ranganathan *et al.,* 2007). In the present study, affected lungs also exhibited chronic inflammatory cells and increased connective tissue proliferation (Fig. 3). Substantial loss of bronchiolar ciliated epithelium accompanied by chronic inflammatory cells and peribronchiolar lymphoid follicle were recorded (Fig. 4). These pulmonary changes could be due to immunological

Table 1. Overall prevalence of *Dictyocaulus viviparous* in fecal samples recorded in cattle and buffaloes

Sex/Species/age	No. of Animal	Positive		95% CI	Odd Ratio/ P value
		N	%		
Male	265	11	4.15	2.20 - 7.10	OR=0.70
Female	180	11	6.11	3.26 - 10.37	[reciprocal =1.42]
Species					
Cattle	210	10	4.76	2.44 - 8.32	OR=0.93
Buffalo	235	12	5.10	2.80 - 8.52	[reciprocal =1.08]
Total	450	22	4.88	3.17- 7.19	-
Age groups					
>1 Year	218	14	6.42	3.70 - 10.29	Mantel-Haenszel chi-sq
<1-3 Year	103	3	2.91	0.75 - 7.72	P>0.210
Above 3Year	134	5	3.73	1.38 - 8.07	

Table 2. Frequency of lung lesions due to *Dictyocaulus viviparus* in buffaloes and cattle

Lesions	Buffaloes (n=5)		Cattle (n=3)	
	N	%	N	%
Gross				
Congested	3	60	2	66.6
Froth in Trachea	1	20	0	0
Consolidation of lungs	2	40	1	33.33
Nodular surface	4	80	3	100
Pleural adhesion	0	0	1	33.33
Purulent exudation	1	20	1	33.33
Parasites in bronchioles	2	40	0	0
Histopathologic				
Developmental stages (Eggs, L1 stage, Adults)	4	80	2	66.66
Mononuclear cells aggregation	5	100	3	100
Congestion	3	60	2	66.66
Septal peribronchiolar fibrosis	4	80	0	0
Alveolitis	4	80	0	0
Peribronchiolar cuffing	3	60	1	33.33

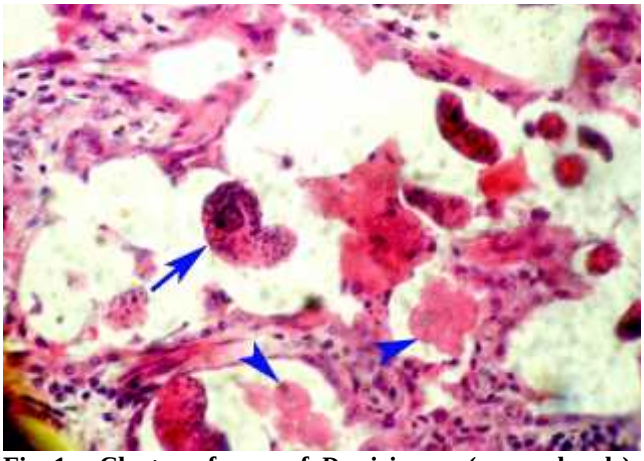

Fig. 1. Cluster of eggs of *D. viviparus* (arrow heads) and newly hatched larvae (arrows). H & E. 400X.

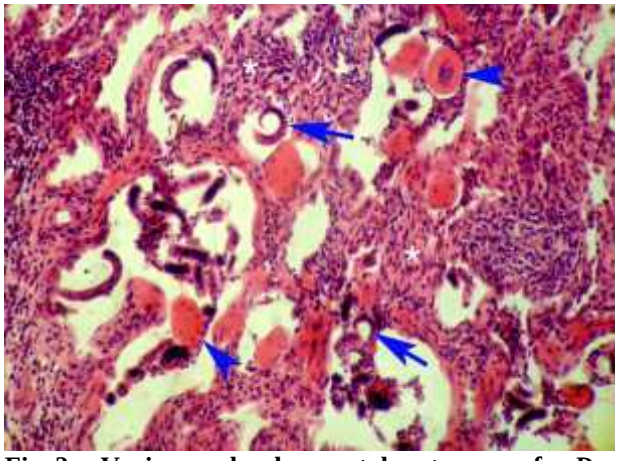

Fig. 2. Various developmental stages of *D. viviparus*, eggs (arrow heads) and adult worms (arrows) along with pneumonic changes and thickened alveolar septa (asterisk). H & E. 200X.

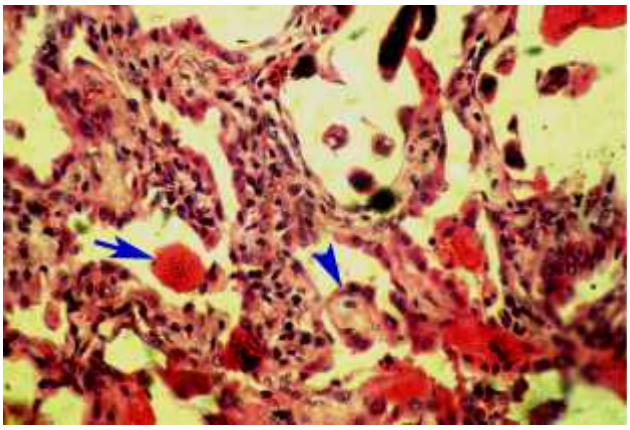

Fig. 3. Lung parenchyma exhibiting the presence of eosinophils, lymphocytes, plasma cells, macrophages and multinucleated giant cells (arrow head) and eggs coated with thick slimy layer (arrows). H & E. 400X.

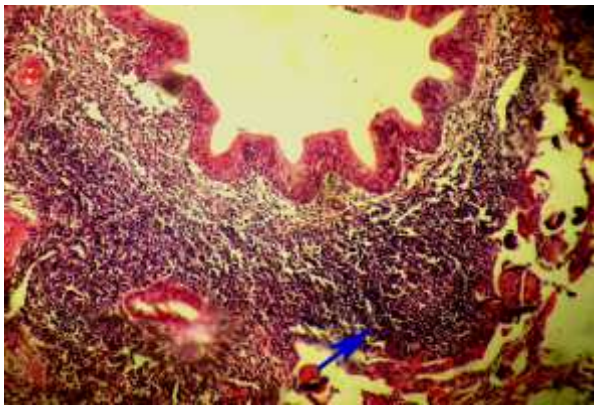

Fig. 4. Extensive mononuclear infiltration around the bronchiole, mainly arranged in nodular fashion (arrow). H & E. 100X.

cellular proliferation in response to the replication of *D. viviparus* eggs and migration of the adult larvae in the lung tissue (Yildiz, 2006). Kooyman *et al.* (2007) reported that *Dictyocaulus viviparus*, the major causative agent of parasitic bronchitis in animals is ingested as larvae and matures into adult worms after penetration of the intestinal wall and via the lymph nodes and blood circulation to the lungs. In lungs, the pathological changes occur due to the influx and activation of eosinophils and mast cells that result in restriction of the airways, resulting in edema, emphysema and collapse of the alveoli. Similar pneumonic changes have also been reported in lungs due to *D. viviparus* infection in cattle (Schnieder *et al.*, 1991), hangul (*Cervus elaphus hanglu*), sheep and goat (Nashiruddullah *et al.*, 2007), foals (Nicholls *et al.*, 1978), red deer calves (Corrigall *et al.*, 1980) and Rocky Mountain elk (Bergstrom 1975).

Conclusions: Previously no study has been conducted to determine the prevalence of *D. viviparus* and its pathology in tropical conditions of Pakistan. The findings of present study are useful predictors of the prevalence of lungworm nematode in cattle and buffaloes. Therefore, further epidemiological and molecular studies be carried for the characterization of this parasite in Pakistan.

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