

PREVALENCE AND ASSOCIATED RISK FACTORS OF AMPHISTOMIASIS IN BUFFALOES FROM PAKISTAN

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ABSTRACT

To investigate the prevalence of amphistome parasites in buffaloes slaughtered at different abattoirs of Rawalpindi and Peshawar district, Pakistan. The gastro-intestinal tracts and bile ducts were examined during the period of May to December, 2017. Out of 198 buffaloes, 39 (12.8%) were infected with multiple species of amphistomes. In present investigation, three species of amphistomes viz *Paramphistomum epiclitum*, *Gastrothylax crumenifer* and *Gigantocotyle explanatum* were identified. The higher infection rate was found in Kundhi breed 13.6% than Azi Khali and Nili Ravi. The males buffaloes (15.1%) and age group of 2 to 4 years (21.4%) had higher infection rate as compared to females and old animals. The mixed infection of amphistomes did not show significant ($P>0.05$) association among breed, sex, age, contact with water bodies and grazing management of animals. The present results will contribute to our understanding of the epidemiology of amphistomiasis in Pakistan for control and prevention studies.

Keywords: *Bubalus bubalis*, buffalo, amphistomiasis, prevalence

INTRODUCTION

In Pakistan livestock sector contributes 11.4% to national GDP and 58.3% of the agriculture sector, and infestation of gastrointestinal helminthes is limiting the productivity of livestock (Ayaz *et al.*, 2013). Amphistomes are digenetic trematodes belonged to four most commonly occurring genera, namely *Paramphistomum*, *Gastrothylax*, *Cotylophoran* and *Gigantocotyle* infecting the rumen and bile duct of buffaloes of Pakistan (Yusuf and Chaudhry, 1970; Soulsby, 1982). Adult flukes inhibiting rumen did not cause much pathogenicity when present in low number, while large number of migrating flukes causes gastroenteritis and bile duct blockage (Halium *et al.*, 2014), the economic losses are in terms of high morbidity, mortality and reduced productivity.

Parasitic co-infections are very common, thus it is a challenge to specifically diagnose them as many trematodes have common reactivity towards certain antigens and diagnosing them through antibody-antigen based tests is difficult, similarly faecal examination cannot be fruitful as many trematodes have similarity in their egg morphology and difficult to distinguish them (Chai *et al.*, 2005). Moreover, egg excretion patterns vary on daily basis and uneven distribution in faces

are some factors hindering the proper diagnosis of trematode infections (Giver *et al.*, 2000). For control and prevention studies on amphistomiasis, it is important to find to the accurate prevalence of these worms infecting the buffaloes. Preliminary studies on prevalence record of mixed amphistomes infection in buffaloes of Pakistan were based on fecal examination techniques and antigen-antibody detection assays, which may lead to miss identification. Therefore, the current investigation was aimed to determine the prevalence of mixed infection of amphistomes collected from rumen and bile duct of slaughtered buffaloes, this approach may reduces the chances of miss identification. The study also investigates mixed amphistomiasis association with respect to breed, gender, age, grazing habit, contact with water bodies and location.

MATERIALS AND METHODS

The entire digestive tracts and bile duct of 198 buffaloes of both sexes (73 males and 125 females) were collected from different slaughter houses of various localities of Rawalpindi and Peshawar district, Pakistan. According to the age, animals were grouped into 2 to 4 years, 5 to 7 years, 8 to 11 and above 11 years age groups. The worms were collected from May to December 2017. Adult amphistomes were removed from buffaloes which were brought to slaughter at local abattoirs. The rumen and bile duct were examined and parasites were removed with the help of forceps, carefully following all precautions to avoid any damage to the parasite. Worms were washed many times with 0.01 M PBS (pH 7.2), stained with borax-carmin and identified using the standard keys (Sey, 1991; Soulsby, 1982). The prevalence percentages and

association of independent variables (sex, age, breed, locality, grazing management and contact with type of water bodies) and infection was evaluated using Chi-Square and Fischer exact test of SPSS software version 20.

RESULT AND DISCUSSION

In current study the prevalence of mixed amphistome infection was 12.1% in buffaloes brought from different districts of Pakistan. Three species of amphistomes *viz.* *Paramphistomum epiclitum*, *Gastrothylax crumenifer* and *Gigantocotyle explanatum* were recorded in this study. The relationship between mixed amphistomiasis and risk factors is given in Table 1. Significant ($\chi^2=31.014$, $P=0.003$) association between geographical areas and infection rate was observed. Highest rate of infection was observed in Gujar Khan (60%), followed by Jhelum (40%), Fateh Jang (21.7%), Charsadda (15.6%), Rawalpindi (13.9%) and Taxila (12.5%). Whereas, mixed amphistomes infection was not recorded from Attock, Hafizabad, Mardan, Nowshera, Peshawar, Sahiwal, Swabi and Talagang. In current study the slaughterhouse survey was performed from May to December that covers the summer, monsoon and post monsoon seasons with sufficient rains and these seasons are favorable for the development of snail intermediate hosts (Hanna *et al.*, 1988), this is the possible reason why we recorded low prevalence rate, as in these months animal picks infection and disease appeared during postpatent period.

Similarly among breeds Kundhi had higher rate of infection (13.6%) as compared to Azi Kheli (12.8%) and Nili Ravi (10.3%), and association was not significant ($P>0.05$) (Figure

1A). Szmidt *et al.* (2000) recorded breed was not significantly associated with amphistome (*P. daubneyi*) infection because some breeds have resistance owing to their genotype (Castelino and Preston, 1979).

The results recorded higher infection rate in males (15.1%) than females (10.4%), and significant ($P>0.05$) association was not found between gender and infection (Figure 1B). The similar findings were observed for paramphistomiasis among slaughtered buffaloes (Javed *et al.*, 2006; Raza *et al.*, 2007). This slightly high infestation in males as compared to females may be explained by the fact that owing to the reproductive importance of female buffaloes they are stall fed during pregnancy and lactation and thus their direct exposure to the pastures is avoided whereas males are set free to graze in pastures near river side and poorly managed (Raza *et al.*, 2007; Javed *et al.*, 2006). Even the female calves are taken better care of as compared to males (Bilal *et al.*, 2009).

Young animals of 2 to 4 years age group showed higher rate of infection (21.4%) as compared to older animals (Figure 1C). The findings are comparable with the results of other studies that showed no significant association between age groups and infection (Khedri *et al.*, 2015). The possible reason of low prevalence in buffaloes older than 4 years of age can be explained by the fact that mature animals develop resistance and strong immunity towards re-infections (Khedri *et al.*, 2015).

In present study animals that were intensive grazers (20%) had highest rate of infection than extensive (15.1%) and semi-intensive (9.1%) grazers (Figure 1D). The results are not in accordance with study that reported significantly high amphistome infection among animals that followed extensive/free range grazing and lesser in intensive grazers

(Biswas *et al.*, 2014).

Mixed infection was more prevalent (18.6%) in buffaloes that interacted with all types of water bodies (Figure 1E). Although animals that interacted with all types of water bodies had the highest rate of infection, possibly because shallow water bodies had more digenetic trematode infection in snails than deep water sources (Jokela and Lively, 1995).

The present study clearly indicates that amphistome infection in buffaloes irrespective of age, sex, breed, grazing management and type of water bodies is a problem that can play a role in hindering the livestock development in the country. Understanding the epidemiology of infection by amphistomes in buffaloes; there is a need for reducing the risk of infection, especially by improving their management to avoid exposure to the gastric trematodes. In order to control of amphistomiasis in this area of Pakistan, the type of intermediate hosts involved in the prevalence of fluke infections should clearly be established in the future.

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Table 1. Relationship of mixed amphistomes infection in slaughtered buffaloes with respect to breed, age, gender, area and grazing management.

Variable	Sample size	Prevalence (%)	χ^2	p-value
Breed			0.434	0.805 ^{NS}
AziKheli	39	12.8		
Kundhi	81	13.6		
Niliravi	78	10.3		
Gender			0.943	0.332 ^{NS}
Female	125	10.4		
Male	73	15.1		
Age			6.537	0.08 ^{NS}
2-4	56	21.4		
5-7	57	7		
8-10	63	9.5		
>11	22	9.1		
Area			31.014	0.003**
Attock	2	na		
Charsadda	32	15.6		
Fateh Jang	23	21.7		
Gujjar Khan	5	60		
Hafizabad	3	na		
Jhelum	10	40		
Mardan	6	na		
Nowshera	10	na		
Peshawar	29	na		
Rawalpindi	36	13.9		
Sahiwal	2	na		
Swabi	14	na		
Talagang	10	na		
Taxila	16	12.5		

Table 1. Relationship of mixed amphistomes infection in slaughtered buffaloes with respect to breed, age, gender, area and grazing management (Continue).

Variable	Sample size	Prevalence (%)	χ^2	p-value
Grazing habit			2.418	0.299 ^{NS}
Extensive	73	15		
Intensive	15	20		
Semi-extensive	110	9.1		
Water bodies			3.805	0.283 ^{NS}
All types	43	18.6		
Canal	70	11.4		
Pond	34	14.7		
Reservoirs	51	5.9		

χ^2 Pearson's chi-square test;

^{NS} non-significant difference ($P > 0.05$);

* Significant difference ($P < 0.05$);

^{na} no infection was recorded

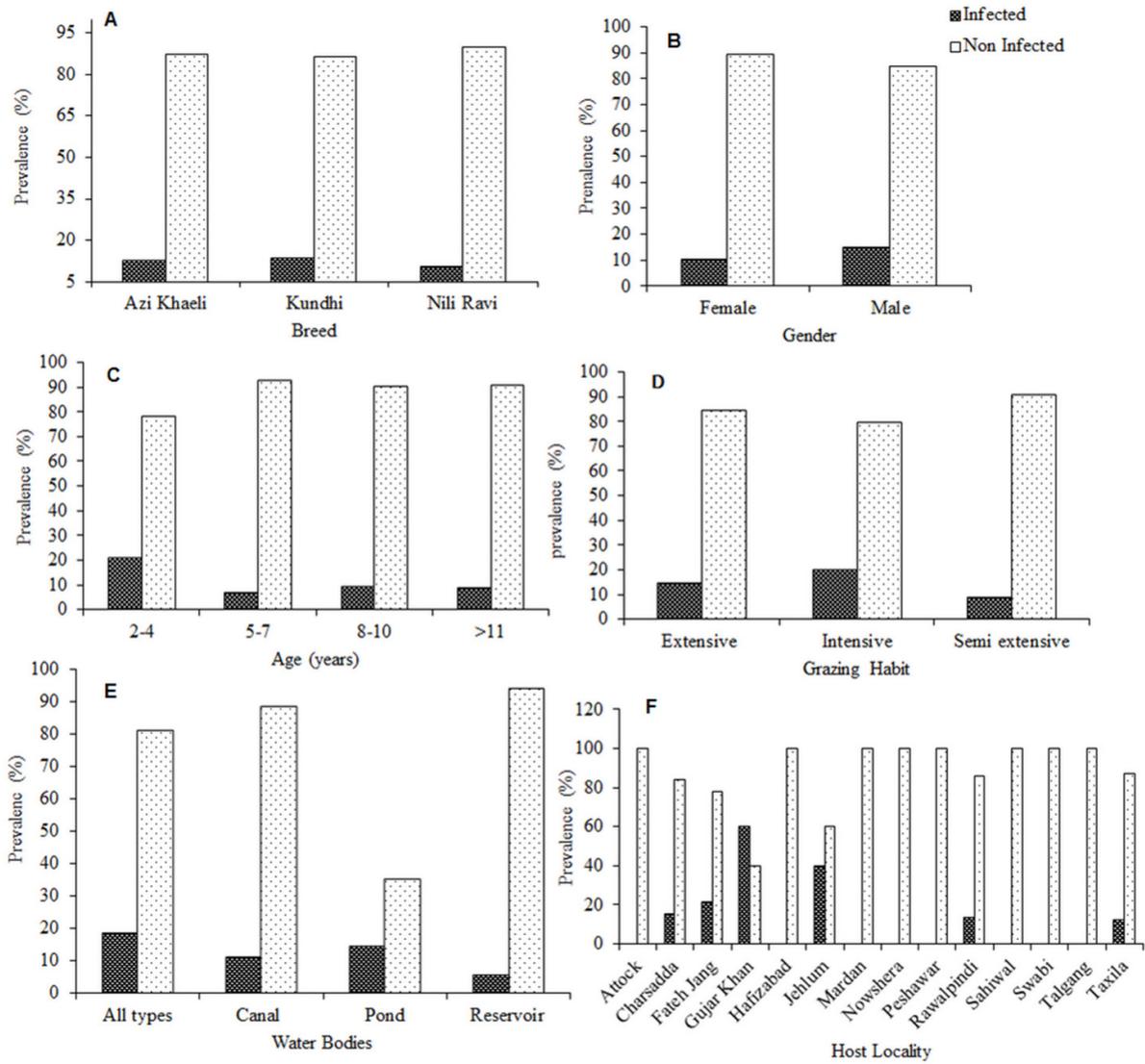


Figure 1. Prevalence (%) of amphiostomiasis in buffaloes among associated risk factors.

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