



Seroprevalence of bovine echinococcus in Pakistan

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ABSTRACT: *Cystic echinococcosis is a parasitic infection of worldwide distribution, which, despite causing significant loss of health and money, is still a neglected disease. The present study was performed to determine the prevalence of bovine hydatid disease at Research Centre for the Conservation of Sahiwal Cattle, Jahangirabad, Khanewal, Pakistan. Five hundred bovine serum samples were tested for the detection of bovine Echinococcus antibodies (IgG), using the ELISA kits. Antibodies of bovine Echinococcus were detected in 24.2% cattle. Female cattle showed higher disease prevalence (33.2%) compared to (6.02%) in male cattle. There was positive association between sex of cattle and prevalence ($P < 0.05$). Positive correlation between disease prevalence and age was detected. Younger animals (<3 years old) had lower prevalence value of disease than to older animals (> 3 years old). It is concluded that there is high degree of exposure of cattle to Echinococcus at Research Centre for Conservation of Sahiwal Cattle, Khanewal, Punjab. Measures should be taken to reduce risk of disease to avoid its spread to humans as well as great degree of economic losses.*

Key words: *Echinococcus antibodies, seroprevalence, ELISA, age, sex.*

Soroprevalência de equinococose bovina no Paquistão

RESUMO: *A equinocose cística é uma infecção parasitária de distribuição mundial que, apesar de causar perda significativa de saúde e dinheiro, ainda é uma doença negligenciada. O presente estudo foi realizado para determinar a prevalência da doença hidatídea bovina no Centro de Pesquisa para Conservação do Gado Sahiwal, Jahangirabad, Khanewal, Paquistão. Quinhentas amostras de soro bovino foram testadas para a detecção de anticorpos anti Echinococcus de bovinos (IgG), utilizando os kits ELISA. Anticorpos de contra Echinococcus bovino foram detectados em 24,2% dos bovinos. As fêmeas apresentaram maior prevalência de doenças (33,2%) em comparação com (6,02%) nos machos. Existe associação positiva entre sexo do gado e prevalência ($P < 0,05$). Foi detectada correlação positiva entre a prevalência da doença e a idade. Animais mais jovens (<3 anos) apresentaram menor valor de prevalência da doença do que animais mais velhos (>3 anos). Conclui-se que existe um alto grau de exposição do gado ao Echinococcus no Centro de Pesquisa para Conservação do Gado Sahiwal, Khanewal, Punjab. Devem ser tomadas medidas para reduzir o risco de doenças e evitar sua propagação para os seres humanos, além de um grande grau de perdas econômicas.*

Palavras-chave: *anticorpos de Echinococcus, soroprevalência, ELISA, idade, sexo.*

INTRODUCTION

The causative agent of hydatid disease is the metacestode stage (larvae) of tape worm of genus *Echinococcus*. It is considered as one of the most harmful parasitic infection in humans and animals around the world (HAYAJNEH et al., 2014). Dog serves as the definitive host for the parasite. While the intermediate host include the great variety of mammals. Definitive host acquire infection by devouring the hydatid cyst present in the viscera of intermediate host. In the definitive host, parasite lives in the intestine where it feeds on the contents of intestine and other visceral organs of final host is not invaded by the parasite. Consequently no

serious harm is convicted to dogs for no lesions (cysts) are formed due to its presence (TORGERSON & BUDKE, 2003). Final host sheds either eggs or gravid segments in feces. Both of them are infective for the intermediate host. In the intermediate host, this disease is identified by a chronic infection due to long term existence of parasite and host responses (cellular and humoral) opposing the infection (AMRI et al., 2007). Resisting responses of the hosts are escaped by the metacestode so that it must not be eliminated from the body. The task is accomplished by the parasite by modification of host responses. Many sorts of immunomodulatory substances are presented to the host by the parasite to hijack the immune responses of the host and to adjust itself within the tissue of host

for the parasite better survival (SIRACUSANO et al., 2008). Varieties of toxic substances are released by cysts and protoscolices. Macrophages of the host are destroyed by these toxic substances and as a consequence assistance is accorded for the formation of hydatid cyst (DE RYCKE et al., 1991).

Meat of infected animal is devalued in both the quality and quantity (the total amount of protein, fats, calcium as well as energy level is low). The protein of such meat has altered amino acids and as a result protein synthesis is damaged and it contains insufficient vitamin B1, B2, A and E. Unsaturated fatty acids (monounsaturated and polyunsaturated) are very low (VALIEVA et al., 2014). Mortality and morbidity of both livestock and humans is the consequence and in endemic areas it has the great societal impacts (CARABIN et al., 2005). Cystic hydatidosis had been categorized as a widespread and very costly disease due to its prevention and treatment by World Health Organization (ADEDIRAN et al., 2014). Food animals infected with this disease present the economic losses in terms of lowered yield of milk, weight loss by the carcass and bad quality of wool. These deficits attribute to serious losses to agriculture for some of them are estimated at $\geq 10\%$ (CARABIN et al., 2005). The gravity of such losses is greatly felt in the developing countries as compared to developed industrialized countries (TORGERSON et al., 2003). Current study was carried out with the aim to sort out the overall prevalence of *Echinococcus granulosus* and its relation to age and gender of host.

MATERIALS AND METHODS

The present study was carried out to determine the overall prevalence of hydatid disease in its relation to gender and age of cattle. Five hundred blood samples from Sahiwal breed of cattle sampled at Research Centre for the Conservation of Sahiwal Cattle, Jahangirabad, Khanewal Punjab (Pakistan), including females and males, were randomly selected during January 2013 to December, 2015. Sample size was chosen according to formula given by COCHRAN (1977) with 95% confidence level. Blood was drawn from the jugular vein of animals. Blood samples were allowed to clot at room temperature for half an hour and serum was obtained after centrifugation of clotted blood. Serum samples were frozen in well labeled serum cups until analysis. Eliza kits supplied by TSZ Elisa (for detection of bovine *Echinococcus* Ig G antibody) were used to test samples for the presence or absence of bovine *Echinococcus* IgG. As per instructions of kit, samples as well as positive and

negative controls were diluted 21 times and added to each well plate. Plates were kept in incubator at 37 °C for 30 minutes. After incubation, plates were washed by aspirating and decanting 200µl to 300 µl of wash solution to each well. Washing was followed by the addition of 100µl of enzyme conjugate to each well and incubation at 37 °C for 30 minutes. Plates were again rinsed 5 times with wash solution and each well was filled with 100µl of substrate solution. Plates were kept in incubator for 15 minutes at 37°C. Incubation was followed by the addition of 100µl of stop solution to each well and within 30 minutes, absorbance was read at 450 nm. The normal range of OD_{negative} was less than 0.21 and OD_{positive} was more than 0.40. Results were calculated by using SPSS version 21. Confidence interval of 95% was used for the overall prevalence, age and for the sex of cattle. Chi-square statistics was used to analyze the association between age groups and prevalence and between sex of host and prevalence.

RESULTS AND DISCUSSION

Present investigation revealed 24.2% (121/500) cattle positive for the presence of *Echinococcus granulosus* antibodies (IgG) through ELISA. The percentage of infection was low in younger animals (age < 3 years) as compared to older one (age > 3 years) (Table 1). Chi square analysis showed positive association between age groups and prevalence ($P < 0.05$). The total 121 animals showing infection of hydatid disease included 111 (33.2%) females and 10 (6.02%) male animals (Table 2). Chi square analysis showed the positive association between male and female cattle for prevalence ($P < 0.05$).

Variations in the values of prevalence for the bovine hydatid disease from different parts of globe have been reported. Most of studies conducted represent abattoir based data. Current findings are similar to investigations of ABUNNA et al. (2011) that reported 23.17% cattle infected with the disease at the municipal abattoir of Nekemte, Ethiopia from 2010 to 2011. Cattle butchered at Tiaret abattoir, Algeria during 2010 had 25.66% prevalence of disease (KOUIDRI et al., 2012). GUADU et al. (2013) reported 25.92% bovines harboring infection at Tigray region of Ethiopia during October, 2010 to December, 2010. Slaughtered cattle in the Delfan region of Iran showed that 25.7% animals had the hydatid disease during 2009 to 2012 (EZATPOUR et al., 2015).

There are some countries around the world who reported a very high percentage of infection

Table 1 - Seroprevalence of *Echinococcus granulosus* in different age categories of Sahiwal cattle at a Research Centre for Conservation of Sahiwal Cattle Jahangirabad of Khanewal, Punjab, Pakistan.

Age (years)	-----Animals-----		-----Prevalence of <i>Echinococcus granulosus</i> -----				
	n	%	-----+ive-----		-----ive-----		95 % CI
			N	%	n	%	
1month-3	164	32.8%	9	5.4%	155	94.5%	0.04,0.063
3.1-6	100	20%	37	37%	63	63%	0.32,0.42
6.1-9	103	20.6%	40	38.8%	63	61.1%	0.34,0.44
9.1-12	49	9.8%	14	28.5%	35	71.4%	0.23,0.35
12.1-15	74	14.8%	18	24.3%	56	75.6%	0.20, 0.28
15.1-18	10	2%	3	30%	7	70%	0.23, 0.33

among bovid and the findings of current investigation seems very low when compared to such data. Example included the findings of ERNEST et al. (2009) that revealed that 47.9% cattle infected with the disease in Arusha, Tanzania during January, 1998 to August, 2008 by observing data records. CHIHAI et al (2012) reported the presence of bovine echinococcosis in 60% animals grazing steppes and flood plains of the Northern Moldova, in an investigation carried at the abattoir of Chisinau. MOJE et al. (2014) reported 50.1% prevalence of disease in cattle butchered in the municipal abattoir of Shashemene, Ethiopia during 2007 to 2008

A very low prevalence value of this disease has declared by from different places throughout the world. For example, a study conducted at the abattoir of Northern India showed 5.39% cattle having of hydatid cyst infection (SINGH et al., 2014). KHAN et al. (2010) indicated a prevalence value of 6.43% in cattle butchered in Lahore, Pakistan from 1999 to 2003. AMER et al. (2018) revealed 2.76% cattle harboring the hydatid cysts in Hail province of Saudi Arabia during 2015. JAWAD et al. (2018) indicated that 1.84% cattle were having infection of hydatid cyst in a butcher shop of Karbala, Iraq during 2016 and 2017.

Multiple factors seem responsible for the observed fluctuations in the values of prevalence in current study and those obtained around the world. It may be associated to the poor hygienic conditions that prevail in different regions and the alarming high number of stray dogs. Remains of animal that die due to any disease become food of such free roaming dogs (TASAWAR et al., 2014). Such remains may contain fertile hydatid cysts, thus infecting stray dogs. These dogs become a major cause of dissemination of infected eggs on pasture. It is not only Pakistan that is facing very high population of such stray dogs, other countries also faced such problem. In a country like Morocco, for example, about 2 millions dogs had been reported (AZLAF & DAKKAK, 2006). Most of the times, these are the guard dogs that roam free during night and day and have close association with the humans and livestock. Their multiplication observed no rule (TADASSE et al., 2014). The major consequence of such free roaming habits of dogs is the contamination of those households where no possession of dogs takes place by the owners. Feeding on rodents and offal by such dogs had been reported (KESTEREN et al., 2013). Most herder were ignorant of the fact that these dogs shed infective stages

Table 2 - Seroprevalence of *Echinococcus granulosus* in male and female sex of Sahiwal cattle at a Research Centre for Conservation of Sahiwal Cattle Jahangirabad District of Khanewal, Punjab, Pakistan.

	Animals		Prevalence of <i>Echinococcus granulosus</i>				
	n	%	-----+ive-----		-----ive-----		95 % CI
			n	%	n	% age	
Male	166	33.2%	10	6.02%	6.02%	93.9%	0.05, 0.07
Female	334	66.8%	111	33.2%	33.2%	66.7%	0.31, 0.35

(eggs) of *Echinococcus* in the field (QINGLING et al., 2014). Having almost no knowledge about mode of transmission of disease, majority of villagers offer raw visceral organs to dogs. These organs may carry infective stage of parasite (ADEDIRAN et al., 2014). Due to financial limitations, rural are unable to purchase drugs meant for the treatment of parasitic infections of dogs (CHINCHULUUN et al., 2014). However, sometimes it is observed that the reason other than financial limitations do not let the treatment of dogs to the owners. One such example is quoted in Nigeria by ADEDIRAN et al. (2014) where dog owners keep the faith that the administration of deworming drugs to their hunting dogs restricts the hunting abilities of dogs. Moreover, such dog owners did not accept the offer of administering free medication to dogs. Religious beliefs of people, in some regions, do not allow the elimination of dogs, as it is observed by Chinese Buddhist (YANG et al., 2009).

Parallel to the problem of large population size of dogs is the carrying out of inappropriate slaughtering practices. Places lacking in proper hygienic facilities are selected for slaughtering purpose. For the domestic use, huge meat quantities are prepared at sites other than abattoirs. In various parties, wedding and religious ceremonies Eid-ul-Azha, animals are slaughtered at home by many families. According to report by AZLAF & DAKKAK, 2006, veterinary inspection is not carried out on animals slaughtered at these occasions. Visceral organs harboring infection are not discarded properly (MOJE et al., 2014). Import of animals may also be associated to the spread of infection. For example, it was reported in Kingdom of Saudi Arabia that the high degree of infection was present among animals imported during pilgrimage season (HAYAJNEH et al., 2014).

Environmental elements also foster transmission of this disease. Altitude variations, for example, influence the floral characteristics of an area thus affecting the grazing habits of animals (HU et al., 2014). Process of pasturing sheep, cattle and other grazing animals on the high-reaching peaks is main practice performed (YANG et al., 2009). Various breed of animals also exhibit difference prevalence of disease. One such example was quoted by BEKELE & BUTAKO (2011). It was observed that local breed of cattle had high prevalence of disease in comparison to the cross breed at municipal abattoir of Wolayita Sodo. Similar observations were also made by ASFAW & AFERA (2014) at Shire municipal abattoir, Ethiopia. However, cross breed of

cattle showed high level of contagiousness than the local breed in the findings reported by TADESSE et al. (2014) at an abattoir house of Nekemte, Ethiopia.

Studies conducted at slaughter houses also poses problems because of the detection of cysts in the offal need detailed examination through multiple incisions. Such practice is not allowed by owners for the infliction of damage to the carcass for detailed examination and decrease the market value of meat and also contaminates it (WANZALA et al., 2003; MOJE et al., 2014). Consequently difference in findings of studies is observed at different abattoirs.

In current study, a high level of epidemicity is noticed in female cattle. Similar observations of greater infection rate among female animals had been noted by various investigators throughout the globe. KASSEM et al. (2013) reported 18.6% female cattle harboring hydatid cyst infection as compared to 12.9% reported in male cattle at abattoir of Sirte, Libya from 2004 to 2005. Similar findings are reported in the slaughter house of Zemen town of Ethiopia during 2013 to 2014 where noticeably higher disease pressure (35.5%) was noted in female cattle in comparison to (26.1%) noted in male cattle (WORKU & NIGATU, 2015). HALEEM et al. (2019) disclosed similar findings of gender wise variation in prevalence of hydatid cyst in various animals being high (25.29%) in females and low (11.97%) in males in Khyber Pakhtunkhwa province, Pakistan (HALEEM et al., 2019).

High degree of infection to hydatid disease noticed in female cattle could be attributed to the number of factors. It is usually observed that the parasitic prevalence and disease expression varies between male and female hosts. Mostly males are prone to get infections as compared to females. However, male showed resistance to infection of certain parasites as compared to females. Such immune reverse condition could probably be due to interaction of endocrine and immune system (KLEIN, 2004). Certain factors can break down the immunity to parasitic diseases in female animals like various stages in the reproductive cycle, lactation and gestation. In these conditions, levels of sex steroid fluctuate which results in altered level of immunity in the host, modification of gene offering resistance to disease, behavior modification influencing the pronicity and immunity of infection (KLEIN, 2000). Physiological and hormonal status of the host provides golden opportunity to the parasite to get establish in these circumstances (DUNEAU & EBERT, 2012). In addition to their effect on the immune response, sex steroid also influence directly the development of

parasite within the host. The female animal eating up the sort of food during lactation process may reduce immunity in them. It was observed during one study that the immunity of mother to various parasitic infections was greatly depressed due to consuming food low in essential amino acids (SAKKAS et al., 2013). Some mechanism may be employed by *E. granulosus* to stimulate testosterone and estradiol production from adrenal gland to create an appropriate environment for the survival within female host and making her more vulnerable to the metacystode of hydatid disease (MOSQUEDA et al., 2007). Parasitic infections showing the sexual dimorphism in infection could also be due to differential exposure by females and male to infective stages of parasite (NAVA- CASTRO et al., 2012). One more reason to elevated prevalence value observed in cows might be due to their longer captivity for breeding that increase the chances for higher prevalence and intensity to the parasitic infections (IBRAHIM et al., 2010). However, bulls are not usually kept longer and have low probability of infection.

Older animals showing elevated prevalence value than younger may be due to the fact that animals can attain infection at any stage of life; however, the appearance of infection in the form of cyst takes time and in older animal they reach to detectable size thus resulting in higher prevalence among older animals (AZLAF & DAKKAK, 2006; IBRAHIM et al., 2010). The longer exposure to parasitic eggs increases the possibility of acquiring infection many fold in older animals. Situation becomes more exasperating because they already have lowered immunity (ADINEHBEIGI et al., 2013). Depressed level of infection in younger animals might be because of their earlier culling or being sold at an earlier age (TEREFE et al., 2012). Most findings round the globe are based on studies conducted at abattoir and chances persist that smaller sized cysts may remain undetected during the examination of carcass of younger animals. Such detection in older animals is easy because cysts get to larger detectable size with the age of host (MUQBIL et al., 2012).

CONCLUSION

Seroprevalence of bovine hydatid disease is high at research centre for conservation of Sahiwal cattle, Jahangirabad. It is emphasizing the need for the proper control program for the hydatid disease. Further research is needed to determine the pathological and economic impact of parasite at the present region.

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DECLARATION OF CONFLICT OF INTEREST

The authors declare no conflict of interest. The founding sponsors had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, and in the decision to publish the results.

AUTHORS' CONTRIBUTIONS

FA performed the experiments. ZT conceived and designed experiments. MHL prepared the draft of the manuscript. All authors critically revised the manuscript and approved of the final version.

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