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RESEARCH ARTICLE

Sero-Survey of Equine Infectious Anemia in Five Draught Equine Populated Metropolises of Punjab, Pakistan

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ABSTRAC

Equine infectious anemia (EIA) is a cosmopolitan fatal recurring retroviral disease of equine family. Keeping in view the importance of the disease, this study was planned and executed to investigate the presence of EIA in 5 draught equine populated metropolises (Lahore, Gujranwala, Faisalabad, Multan and Bahawalpur) of Punjab, Pakistan. A cross-sectional epidemiological survey was conducted and 430 blood and serum samples were randomly collected from 332 donkeys, 65 horses, and 33 mules along with epidemiological information. Samples were then transported to the laboratory for analysis. Blood samples were tested to determine erythrocyte indices for establishing the type of anemia. Serum samples were analyzed for the presence of antibodies against EIA virus through a commercial ELISA. Although, erythrocyte indices indicated towards presence of anemia in equines, no serum sample was found positive on ELISA. This is a first ever study in Pakistan where presence of EIA was investigated over a wide geographic region and indicated towards the possible disease free status of the selected equine population.

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INTRODUCTION

Draught equines are versatile breeds known for their strength, stamina, patience and docile temperaments. These animals are extensively used for various purposes in the developing economies including farming and pneumatic cart pulling. The total equine population of Pakistan is 4.7 million comprising of 4.2, 0.15 and 0.34 million donkeys, mules and horses, respectively. Out of this, approximately 33.3, 64.7 and 60.6% donkeys, mules and horses, respectively are kept for draught purpose (Anonymous, 2006).

Equine infectious anemia (EIA) is a potentially fatal disease of *equidae* caused by a lentivirus belonging to family *Retroviridae* which is a close relative to human immunodeficiency virus (HIV) (Nakajima and Sugiura, 1994). Clinically, EIA is a persistent infection with recurrent cycles of viremia and episodic fever, anemia, edema and loss of weight (Sellon, 1993; Radostits *et al.,* 2007). Anemia, thrombocytopenia, hypergammaglobinemia and elevated liver enzymes are common hematological and biochemical findings in clinical disease (Cullinane *et al.,* 2007). Affected equines often recover and remain carriers throughout their lives. Recovered animals may suffer from recurring clinical (chronic) disease caused by new mutant EIA virus strains with the signs of fever (105-108°F), petechial hemorrhages, depression, weight loss, dependent edema, progressive non regenerative anemia (Sellon, 1993; Riegel and Stockham, 2010) and are often termed as a "swamper" (Clabough *et al.*, 1991). EIA is more detrimental in its inapparent form and poses the real threat to other healthy equines. Clinical signs are absent or negligible in carriers and they suffer from constant viremia. If undetected these equines may spread infection to others through biting insects and hypodermic needles etc. (Albayrak and Ozan, 2010). No seasonal, sex or age based association could be ascertained on the basis of previous studies (Sellon, 1993; Radostits *et al.,* 2007) and no vaccine or treatment is recommended in equines (Albayrak and Ozan, 2010).

Prior to the development of a reliable ager gel immunodiffusion (AGID) test also known as Coggins test, sero-diagnosis of EIA was a scientific dilemma (Issel and Cook, 1993). AGID detects the precipitating antibodies against group reactive and antigenically stable core protein p26 (Piza *et al.,* 2007). However, detection of EIA in equines suffering from first bout of disease and foals born to test positive dams is major limitations of the

AGID (Issel and Cook, 1993). Efforts to find a faster and rapid serological test for screening resulted in the development of ELISA in late 1980s and currently more sensitive competitive ELISA (cELISA) are commercially available to detect antibodies against core p26 protein. Investigators have found a good correlation between AGID and cELISA results (Soutullo *et al*., 2001; Parė and Simard, 2004; Piza *et al*., 2007). The ELISA is a rapid and easier to interpret test (Cullinane *et al.,* 2007) that has been used for screening and surveillance studies in recent years (Gill *et al.,* 2008; Albayrak and Ozan, 2010). However, ELISA is less specific and positive sera should be confirmed through AGID (Parė and Simard, 2004). Whenever rapid test results are needed, disease status is unknown and equines are present at remote location, the ELISA becomes the test of choice (Piza *et al*., 2007). Furthermore, ELISA can also be employed as an international pre-movement screening test because of its sensitivity and rapidity (Cullinane *et al.,* 2007). Despite the considerable efforts put in to control EIA by the developed nations, the challenges posed by EIA are still unrequited. For instance, from 1980 to 2005 equine owners of America spent 600 million dollars for testing and control of EIA that highlights the personal and financial efforts required to control the disease (Issel *et al.,* 2005).

Since the first description of EIA in 1843, it has been reported worldwide and during past 5 years (2006-2010) disease has been reported from 44 countries (Anonymous, 2011). It is feared that actual incidence of EIA is more than estimated, as many countries are still not conducting surveillance studies. A continuous surveillance is required (Bicout *et al.,* 2006) to acquire and maintain the disease free status in a country (Turan *et al.,* 2002; Ataseven and Arslan, 2005; Kirmizigül *et al.,* 2009). EIA was first reported in neighbouring India at Karnatika in 1987 (Uppal and Yadav, 1989) and later incidence was also recorded in the states of Maharashtra, West Bengal, Haryana, Delhi and Indian Punjab (Singh *et al*., 1997). In Pakistan, a solitary study regarding EIA indicated towards the absence of disease in equines of Faisalabad metropolis (Gill *et al.,* 2008). Presence of disease in neighbouring India, existence of vectors (mosquito and fly) in Pakistan and sparse efforts on part of local investigators led to the planning of presented study to investigate the presence of EIA in draught equine population over a wider geographic area.

MATERIALS AND METHODS

A cross-sectional serological survey was planned to collect blood and serum samples from 5 draught equine populated metropolises of Punjab (Lahore, Faisalabad, Gujranwala, Multan and Bahawalpur). Geographic and climatic detail along with equine population of the selected cities is presented in Table 1. Sample size was calculated on the basis of expected prevalence of disease to be 50% (unknown) at 95% confidence level and 5% desired absolute precision (Thrusfield, 2005). The metropolises were selected based upon draught equine population and community network of Brooks Hospital for Animals, Pakistan to ensure the cooperation of equine owners. In total 430 samples (blood and serum) from 332 donkeys, 65 horses and 33 mules of either sex and various age groups along with epidemiological information were randomly collected and transported to postgraduate laboratory of Department of Clinical Medicine & Surgery. Distribution of samples collected from selected areas accompanied with species and sex breakdowns is presented (Table 2).

Blood samples were subjected to hematological examination as described by Gul *et al.* (2007) and erythrocyte indices were calculated to classify the type of anemia (Thrall, 2004). Serum was collected and stored at -40°C till further processing. Serum samples were tested through a United States Department of Agriculture (USDA) approved commercial ELISA of VMRD, Inc., Pullman, USA (Susan *et al.,* 2008) by strictly following the recommendations of the manufacturer. After completion of the recommended steps the plates were first subjected to visual observation then read at 450 nm and optical density (OD) values were recorded. A sample was considered positive if the test well yielded color development and OD value equal to or greater than positive control serum. Hematological values were compared with those documented for the local equines (Gul *et al.,* 2007). Data was further analyzed through one way analysis of variance (ANOVA) and unpaired t-test through IBM SPSS Statistics 17.0 for Windows®, IBM Corporation, Route 100 Somers, New York, USA.

RESULTS

Hematological values regarding red blood cell (RBC) counts, hemoglobin (Hgb) concentrations and hematocrit (HCT) suggested the presence of anemia in apparently healthy sampled equines (Table 3). RBC counts were lower ($P>0.05$) than the respective reference values in horses, mules and donkeys. Mean hemoglobin (Hgb) concentration values were found decreased than reference in all equine types and this difference was significant in horses ($P<0.001$). Hematocrit values were also significantly lower ($P<0.001$) than the reference values in horses, mules and donkeys. Erythrocyte indices indicated towards presence of microcytic hyperchromic type of anemia in horse, mules and donkeys. However, all tested sera from 5 sampled metropolises failed to mount a response for EIA virus antibodies upon ELISA test and thus were considered negative for equine infectious anemia.

DISCUSSION

In the areas under study, draught equine population was dominated by donkeys followed by horses and mules. This high donkey population was due to the economical limitations of owners, less feeding and space requirements by these animals along with more docile temperament. The draught equines in Pakistan usually exist in sub-optimal productive stage owing to stress caused by draught work, overcrowding, malnutrition and diseases (known and unknown). Moreover, relatively higher intra-animal contact in working equine communities favors the chances of spread of disease in these populations.

Although the hematological values pointed towards presence of anemia in equines but the values were not indicative of progressive non regenerative anemia found in

Table 1: Geographic and climatic details along with equine population of the 5 draught equine populated metropolises of Pakistan sero-surveyed for the presence of equine infectious anemia

Metropolis	Elev.(ft)	Geographic Coordinates		Average Temperature				Equine Population		
		Lat.	Long.	Summer °C		Winter °C				
				Av. Max.	Av. Min.	Av. Max.	Av. Min.	Horse	Mule	Donkey
Lahore	712	31.55	74.34	36	25	19	7	4596	721	17386
Faisalabad	600	31.4	73.04	39	27	21	6	6645	3046	44144
Gujranwala	744	32.16	74.19	38	24	21	6	4612	1841	18964
Multan	710	30.18	71.47	40.3	23	27	5.8	1940	954	19076
Bahawalpur	370	29.37	71.68	40	28	30	14	1238	954	19076

Table 2: Distribution of samples along with species and sex collected for the sero-survey of equine infectious anemia in 5 draught equine populated metropolises of Punjab, Pakistan

Area Sampled	Horses Tested (sex)	Mules Tested (sex)	Donkeys Tested (sex)	Total
Faisalabad	18 (5♂,13♀)	9 (6♂,3♀)	123 (98♂,25♀)	150
Lahore	13 (4♂,9♀)	6 (3♂,3♀)	50 (34♂,16♀)	69
Gujranwala	14 (5♂,9♀)	6 (4♂,2♀)	55 (43♂,12♀)	75
Multan	10 (6♂,4♀)	6 (3♂,3♀)	52 (37♂,15♀)	68
Bahawalpur	10 (5♂,5♀)	6 (2♂,4♀)	52 (40♂,12♀)	68
Total	65 (25♂,40♀)	33 (18♂,15♀)	332 (252♂,80♀)	430

Table 3: Comparison of hematological values recorded in horses, mules and donkeys sampled for sero-survey of equine infectious anemia in 5 draught equine populated urban areas of Punjab, Pakistan with those reported by Gul *et al.* (2007)

Parameter	Horses		Mules		Donkeys	
	Tested (n=65)	Reported Values (n=242)	Tested (n=33)	Reported Values (n=20)	Tested (n=332)	Reported Values (n=153)
WBC ($X10^9$/L)	6.65±2.16	7.18±2.87	6.82±2.12*	9.40±2.89	6.77±1.91*	9.75±3.29
RBC ($X10^{12}$/L)	5.28±1.18	5.93±2.81	5.77±1.68	5.74±2.62	5.78±1.56	5.88±2.65
Hgb (g/dL)	9.62±2.18*	10.93±1.81	9.24±2.05	10.10±0.73	9.25±1.82	9.01±1.13
HCT (%)	28.59±5.30*	37.75±4.57	28.86±5.36*	34.37±4.13	29.22±5.48*	32.42±4.38
MCV (fl)	56.30±14.65	-	53.48±16.34	-	54.30±18.37	-
MCH (pg)	19.82±7.28	-	16.93±5.29	-	17.09±5.50	-
MCHC (g/dL)	34.17±7.57	-	32.67±7.80	-	30.56±8.62	-

WBC: white blood cell count, RBC: red blood cell count, Hgb: hemoglobin concentration, HCT: hematocrit, MCV: mean corpuscular volume, MCH: mean corpuscular hemoglobin, MCHC: mean corpuscular hemoglobin concentration. *Values are significantly different ($p<0.01$) from the respective reference values.

EIA (Sellon, 1993; Riegel and Stockham, 2010). Instead these values indicated the presence of microcytic hyperchromic sort of anemia possibly pointing towards chronic infections, malnutrition and iron deficiency (Thrall, 2004).

The sampled draught equine population of the studied metropolises found to be free from antibodies against equine infectious anemia virus on the basis of ELISA. The commercial ELISA (VMRD, Inc, USA) used in current study detects the antibodies against EIA virus p26 (static region) gene in affected equine sera. The test has well established sensitivity (100%) and specificity (100%) by different laboratory (Susan *et al.,* 2008) and field studies (Cullinane *et al.,* 2007) and is approved by the United States Department of Agriculture (USDA) for the field and laboratory diagnosis of EIA. This finding is in accord with results reported by Gill *et al.* (2008) as they found no sero-reactive animal for EIA in randomly selected equines of Faisalabad region that may be ascribed to the absence of the disease or failure of the organism to mount a response in the sampled equine population. Similar findings have been noted in the preceding studies elsewhere (Turan *et al.,* 2002; Ataseven and Arslan, 2005; Kirmizigül *et al.,* 2009; Mashhadi *et al.,* 2010).

Although, the study pointed towards the possible EIA free status of the draught equines from the selected metropolises, further serological and molecular investigations focusing new locales and wider sample size are required to strengthen this claim in Pakistan.

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