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

M. Hammad Hussain*, Ghulam Muhammad, Ijaz Javed1 and Muhammad Siddique2

Department of Clinical Medicine & Surgery; 1Department of Physiology and Pharmacology; 2Department of Microbiology, University of Agriculture, Faisalabad, Pakistan; *Deceased

* Corresponding author email address: m.hammad.hussain@gmail.com

ARTICLE HISTORY
Received: August 15, 2011
Revised: November 21, 2011
Accepted: November 24, 2011

Key words:
EIA
ELISA
Equines
Pakistan
Sero-survey

ABSTRACT
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.

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; 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). 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
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). Geographical 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 intranimal 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

MATERIALS AND METHODS

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; Paré 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 (Paré 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 unrequited. For instance, from 1980 to 2005 equine owners. In total 430 samples (blood and serum) from 332

chances of spread of disease in these populations. 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 intranimal 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

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). Geographical 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 intranimal 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
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 seroreactive 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; Kirmizigil 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.

Acknowledgement: Authors are thankful to the Brooke Hospital for Animals, Pakistan for facilitating the field sampling in their communities of Lahore, Faisalabad, Gujranwala and Multan metropolises.

REFERENCES


Thrall MA, 2004. Veterinary Hematology and Clinical Chemistry. Lippincott Williams & Wilkins, Philadelphia, USA.

Thrusfield M, 2005. Veterinary Epidemiology. 2nd Ed, Blackwell Science Ltd. UK.
