Acta Tropica 180 (2018) 18–25

Contents lists available at ScienceDirect

Acta Tropica

journal homepage: www.elsevier.com/locate/actatropica

First molecular evidence of equine granulocytic anaplasmosis in Pakistan

Sehrish Saleem[a], Muhammad Ijaz[a,*], Shahid Hussain Farooqi[a], Muhammad Imran Rashid[b], Amjad Khan[c], Awais Masud[d], Amjad Islam Aqib[a], Kashif Hussain[a], Khalid Mehmood[e], Hui Zhang[f,*]

[a] Department of Clinical Medicine and Surgery, University of Veterinary and Animal Sciences, 54600 Lahore, Pakistan
[b] Department of Parasitology, University of Veterinary and Animal Sciences, 54600 Lahore, Pakistan
[c] Department of Epidemiology and Public Health, University of Veterinary and Animal Sciences, 54600 Lahore, Pakistan
[d] District Diagnostic Laboratory, Livestock and Dairy Development Department, 42200 Mianwali, Pakistan
[e] University College of Veterinary and Animal Sciences, Islamia University of Bahawalpur, Pakistan
[f] College of Veterinary Medicine, Huazhong Agricultural University, Wuhan 430070, People's Republic of China

ARTICLE INFO

Keywords:
Equine granulocytic anaplasmosis
Anaplasma phagocytophilum
Phylogenetic analysis
Risk factors
Hematological parameters

ABSTRACT

Anaplasma phagocytophilum (*A. phagocytophilum*) is an obligate intracellular bacterium that causes equine granulocytic anaplasmosis (EGA) disease in equines. This pathogen has zoonotic potential, which makes it very important to be detected and controlled as early as possible. This study was aimed to assess the molecular prevalence, associated risk factors of EGA along with its effects on various hematological parameters. This study revealed an overall 10.67% prevalence in equine. Horses showed highest prevalence followed by mules and donkeys presenting 11.86, 10.53 and 9.43% prevalence, respectively. The samples were confirmed for anaplasmosis through sequencing. The BLAST queries confirmed very high homology of our isolates with Chinese and Japanese isolates of *A. phagocytophilum* (Accession no's; KX505303, KY242456 and LC002836). The phylogenetic analysis found the study isolates clustered with each other and this cluster closely resembled Chinese isolate of *A. bovis* (FJ169957), *A. phagocytophilum* (HQ872464) and *A. phagocytophilum* (NR_044762) human isolate from northern Minnesota and Wisconsin. The key risk factors identified for occurrence of EGA in equine species on the basis of univariable analysis were sex of animal, housing type, tick infestation, previous tick history and tick control status, type of acaricides used, rearing system and farm hygiene, respectively. The hematological parameters like Hemoglobin (Hb), Total Leukocyte Count (TLC), Total Erythrocytes Count (TEC), and granulocytes were decreased in diseased animals. The mules showed no typical hematological variations which make sense for its nature as carrier of infection to the susceptible species. This is the first molecular evidence of EGA in Pakistan. The disease needs to be handled seriously as it has zoonotic potential. The animals should be properly attended in disease conditions as leukopenia, neutropenia and lymphopenia can aggravate the condition by making the animal prone to secondary infections.

1. Introduction

The equine population in Pakistan has risen from 4.8 million in 2006 to 5.7 million (asses 5.1, mules 0.2, and horses 0.4) as per the economic survey report in 2015–2016. Over 60% of all horses and 95% of all mules and donkeys are found in developing countries (Fielding, 1991), which plays a key role in their economics (Pearson et al., 2005). In Pakistan, equines are raised as draught animal for transportation, riding, racing and companion animal. However, in urban and rural areas of Pakistan the role of equine as working animal is more crucial (Javed et al., 2014).

Equines are susceptible to different medical ailments likewise other animal species that are of great economic importance to the equine owners (Goraya et al., 2013), which results in ill health and calamities in case of severity (Chaudhry et al., 2014). Ticks are the most important ectoparasites that are not only responsible for direct damage and blood loss but also the transmission of various diseases such as equine lymeborreliosis, piroplasmosis and equine granulocytic anaplasmosis (Sigg et al., 2010). Equine tick borne hemoparasitic diseases, affecting mules, donkeys, horses and zebras are most commonly manifested as acute hemolytic conditions in the affected species (Traub-Dargatz et al., 2010).

Anaplasmosis caused by *A. phagocytophilum*, a pathogen infecting many species of domesticated and wild mammals, including equines

* Corresponding authors.
E-mail addresses: mijaz@uvas.edu.pk (M. Ijaz), ahstuzh@sina.com (H. Zhang).

https://doi.org/10.1016/j.actatropica.2017.12.032
Received 27 October 2017; Received in revised form 29 December 2017; Accepted 29 December 2017
Available online 05 January 2018
0001-706X/ © 2017 Published by Elsevier B.V.

(Passamonti et al., 2010). Equine granulocytic anaplasmosis (EGA) is a multi-host infectious disease in human and animal that is characterized by thrombocytopenia (Chan et al., 2010). The disease prevails in high tick activity season i.e. spring and autumn (Bown et al., 2003; Rymaszewska and Grenda, 2008). The etiological agent is a gram-negative, small, pleomorphic or spheroid shaped bacteria which are reported to reside principally inside thegranulocytes and especially neutrophils in infected animal (Björsdorff et al., 2002; McQuiston et al., 2003).

To date, only few microscopic studies are conducted regarding the prevalence and hematology of EGA in Pakistan (Javed et al., 2014; Razzaq et al., 2015). PCR is considered as the gold standard for the diagnosis of anaplasmosis (de Echaide et al., 2005) but unfortunately PCR based diagnosis is still not developed in many areas of Pakistan. Previously conducted studies regarding the risk factors associated with equine anaplasmosis were confined to small areas in Pakistan (Razzaq et al., 2015). It is very important to obtain correct information about the prevalence and molecular diagnosis of equine anaplasmosis as the etiological agent has zoonotic potential.

This study is the first molecular based evidence of EGA presence in Pakistan. This study also focuses on association of various hypothesized risk factors with EGA and effects of this malady on various hematological parameters of equines.

2. Material and methods

2.1. Sampling strategy

This study was conducted from March to August 2017. The target subjects were 150 diseased equines (59 Horses, 38 Mules and 53 Donkeys) from district Lahore located in the northeast of Punjab, Pakistan (Fig. 1). Animals showing clinical signs such as; fever, anorexia, lethargy, icterus, petechiae, reluctance to move, distal limb edema and ataxia were included in the study. Every 3rd diseased animal presented at various public and private hospitals was included in the study. The majority of animals were originated from small holder farmers, keeping equine as a draught animal.

The selected equines were examined clinically for the primary screening as study subjects. Afterwards, thin blood smears were made in triplets from the ear tips for the screening of animals through microscopy of Giemsa-stained blood smears as described by Moretti et al. (2010) to observe intracellular bodies, resembling Anaplasma. Besides smear microscopy 2 ml of blood was drawn into EDTA coated vacutainers for DNA extraction using appropriate extraction kit. The blood samples were transported to Medicine laboratory, University of Veterinary and Animal Sciences, Lahore maintaining the cold chain using iceboxes. Each sample was accompanied by a piloted questionnaire for capturing the information regarding animal, management and environmental factors.

2.2. DNA extraction

DNA was extracted from all the blood samples using genomic DNA extraction kit (GeneAll®, Exgene™, Cat. No. 105-101). All the steps in DNA extraction were carried out according to the manufacturer's instructions. The extracted DNA from all the samples was checked by using gel electrophoresis to monitor whether sufficient DNA has been extracted. The DNA was stored at −20 °C till analysis by PCR.

2.3. Molecular screening for Anaplasmaspps

The molecular screening was based on amplification of 16S rRNA of *Anaplasma* spps. The primers reported by Parola and Raoult (2001) were utilized to amplify 345 bp fragment of 16S rRNA gene of *Anaplasma* spps. The primers set consisted of forward primer EHR 16SD: 5′-GGTACCYACAGAAGAAGTCC-3′ and reverse primer EHR 16SR: 5′-TAGCACTCATCGTTTACAGC-3′. The PCR mixture was prepared in a final volume of 20 µl consisting of 10 µl of TOP real™ GeneAll® (GeneAll Biotechnology Co., Ltd.) qPCR 2x Pre MIX (containing 0.2 U of Taq/µl), 2 µl of DNA sample and 2 µl (10 pmol) of each primer. Reaction was

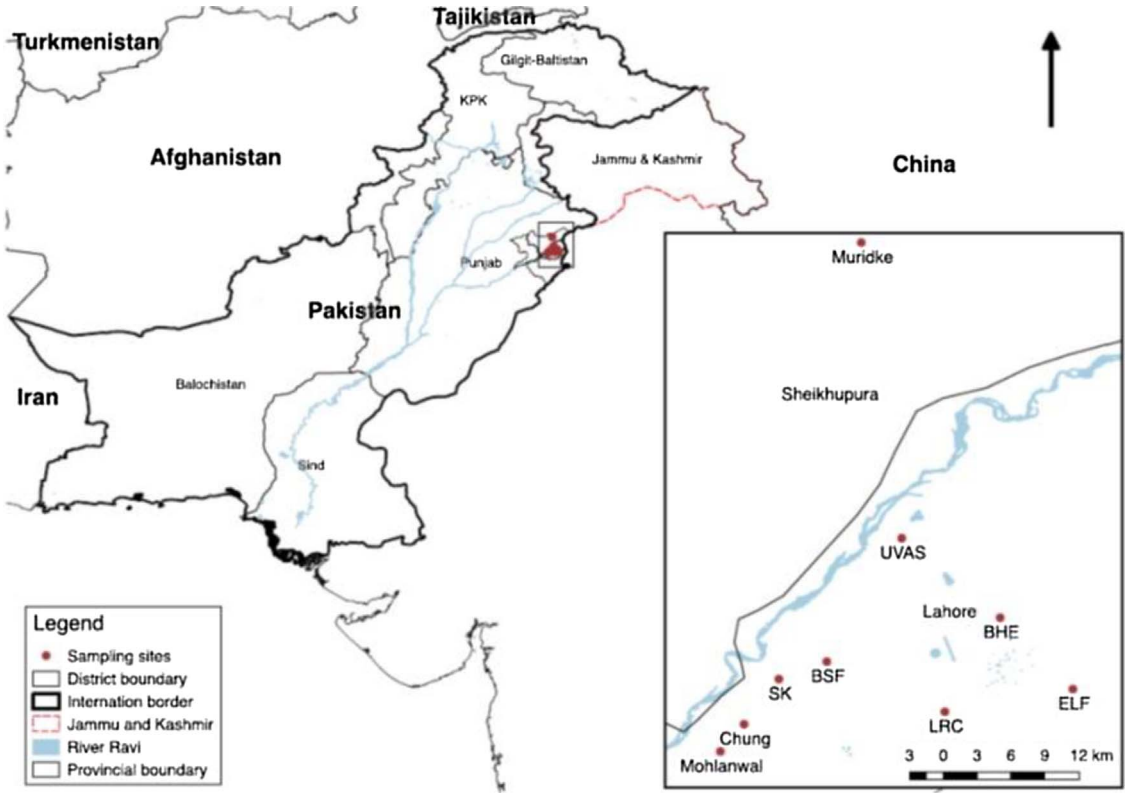

Fig. 1. GIS map of study area showing sampling regions of district Lahore.

Download English Version:

https://daneshyari.com/en/article/8744378

Download Persian Version:

https://daneshyari.com/article/8744378

Daneshyari.com