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FACTORS INFLUENCING THE INCIDENCE OF *EIMERIA LEUCKARTI* IN HORSES

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ABSTRACT

Cross sectional study was planned to figure out the prevalence rate of *Eimeria (E.) leuckarti* in horses and factors influencing its occurrence in district TT Singh, Punjab, Pakistan. From April 2009 to March 2010, equine feces were collected. Two stage cluster method was adopted for random selection of horses. Total of 484 fecal samples were collected from whole district and collected samples were subjected to quantitative floatation method. It was found that 50.41% of sampled horses were positive for *E. leuckarti* with prevalence reaching to its peak in August (OR=1.156; χ^2=20.055) while the lowest occurrence was recorded in April, May and June, coinciding with the period of lowest humidity and precipitation of the year in Pakistan. It can be inferred from these observations that wet season seems to be favorable for propagation of this disease. Foals (124/197; 62.94%; OR=0.422; χ^2=20.825) were found to be more susceptible ($P<0.05$) to *E. leuckarti* in comparison to adults (120/287; 41.81%) while mares with prevalence of 56.48% (196/347; OR=0.512; χ^2=13.265) were found to be more susceptible ($P<0.05$) as compared to and male horses (48/137; 35.04%). Horses kept on non-cemented floor, mix farming with other animals, given food on ground and watered in ponds/common places were found to have higher rates of prevalence of *E. leuckarti* ($P<0.05$) in comparison to those kept on cemented/partially cemented floor, separate farming, food in trough and tap watered, respectively. It is inferred from findings of this survey that disease can be controlled by improving management systems.

Keywords: *Eimeria Leuckarti*, Epidemiology, Equine, Pakistan

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1. INTRODUCTION

Equines are used for multipurpose in developing world. About two third of equine diseases are comprised of gastrointestinal diseases. Coccidiosis is an important disease that lowers the performance of horses. Species of Eimeria responsible for coccidiosis in horses include *E. leuckarti*, *E. solipedum* and *E. uniungulsti* (Ghahfarrokhi et al. 2014). *Eimeria leuckarti* was first named after the name of Rudolf Leuckart (Dubey and Bauer 2018). Dubey and Bauer (2018) stated that *E. leuckarti* is the only species responsible for coccidiosis in horses. Eimeria is an intracellular parasite of phylum Apicomplexa of Kingdom Protista which infects intestine. *Eimeria leuckarti* enter epithelial cells of intestine and then they penetrate to lamina propria (Hirayama et al. 2002). Signs and symptoms of coccidiosis in horses include acute and chronic diarrhea, acute abdominal pain, colic and uthrifty (Dubey and Bauer 2018). Infection is higher in foals as compared to adults. Foals can get oocysts of *E. leuckarti* as early as first day after birth (Dubey and Bauer 2018).

There are various reports of coccidiosis in domestic animals of Pakistan (Rehman et al. 2012; Khan et al. 2013) but there is not enough data on coccidiosis in horses of Pakistan. Moreover, there is scarce information on epidemiological factors affecting incidence of coccidiosis in horse. As the drug resistance has been increasingly reported, research interests of scientific community have been shifted to alternative ways that may comprehend use of plants, determinants influencing prevalence, use of probiotics (Abu-Akkada and Awad 2015) and search of novel vaccine targets (Khan et al. 2016). Deep understanding of epidemiology of coccidiosis in horses may assist in effective control and prevention of disease.

2. MATERIALS AND METHODS

Fecal samples of horses were collected from whole district of Toba Tek Singh. Sampling technique followed for random sampling was two stage cluster sampling with primary units being union councils. Sample size was determined through following formulae (Thrusfield 2008).

As there was no information available about prevalence of disease in study area, expected prevalence was considered 50%. Using formula given below, samples were collected from 45 union councils out of total 82 union councils. Map grid method was used for selection of union councils. Number of primary clusters i.e., union councils were determined using following equation.

$$g = \frac{1.96^2\, T_s\, V_c}{d^2 T_s - 1.96^2\, P_{exp}\,(1 - P_{exp})}$$

A pilot survey was done in three councils to calculate between cluster variance. Number of sampled horses was calculated by following formula.

$$n = \frac{1.96^2\, P_{exp}\,(1 - P_{exp})}{d^2}$$

A questionnaire comprising of dichotomous and multiple-choice questions was predesigned and further refined after pilot survey for collection of information regarding risk factors influencing the incidence of disease. Information regarding following variables were collected.

Age: Sampling animals were divided into two categories of foals and adults for collection of feces. Animals of less than one year age were categorized as foals and older than four years as adults.

Sex: Feces of both sexes were collected. About fifteen grams of feces from 3 different fecal balls were collected for preservation in potassium dichromate (2.5%). Fecal samples were then carried to laboratory. Fecal samples were examined through floatation technique using saturated solution of zinc sulphate following method of Zajac and Conboy (2006) with slight modification. Oocysts were then identified by morphological features as described by Soulsby (2006). To determine the effect of relative humidity, rainfall and temperature on occurrence of disease, meteorological data was provided by Meteorological cell, Department of Crop Physiology, University of Agriculture, Faisalabad.

Mantel–Haenszel (MH) chi-square and Odds Ratio (OR) were used to analyze the effect of factors with paired characteristics. SAS was used to carry out all statistical procedures (SAS 2003).

3. RESULTS

In this study 484 samples were collected and examined for presence of *E. leuckarti*. *Eimeria leuckarti* was only species which was identified in horses of T.T. Singh. It was found that 50.41 percent of horses were positive for *Eimeria leuckarti*. Statistical analyses (OR & Chi-square) showed that prevalence of disease is significantly influenced by sex and age of horse, floor type, feeding system and farming system. Highest prevalence (74.36%) was found in month of August (rainy season) (Fig 1). Fig. 1 shows a relation between rain fall, temperature, relative humidity, and occurrence of Eimeria. Prevalence of Eimeria seems to be high after highest amount of precipitation. Strong negative correlation was found between prevalence of disease and age of horse.

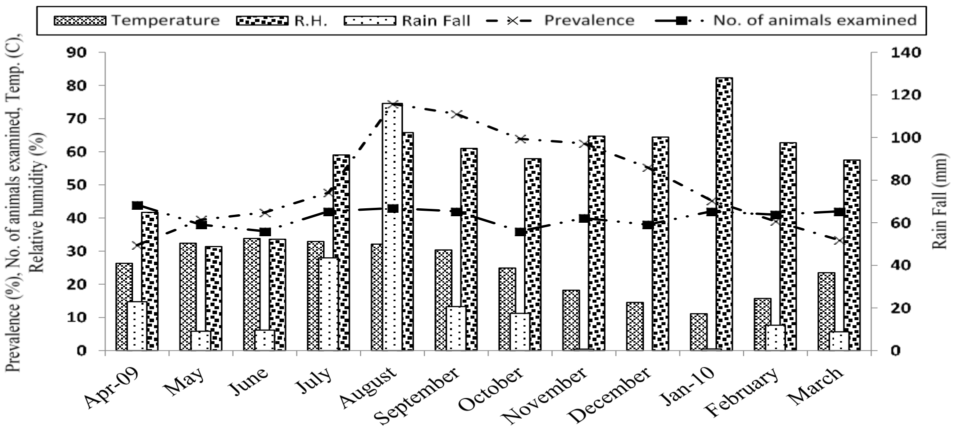

Fig. 1: Prevalence of *Eimeria* in horses of Toba Tek Singh district, Pakistan during changes in rain fall (mm), temperature and relative humidity in the period April 2009 to March 2010.

Higher prevalence was recorded in foals (62.94%) in comparison to adults (41.81%; OR=0.422; χ^2=20.8252) (Table 1; Fig. 2). Oocyst discharge in feces of foals was found to be significantly higher as compared to those in adults. Significantly lower oocysts per gram (OPG) was recorded in feces of adults (χ^2=11.3257; OR=2.012) in comparison to foals. Lower prevalence was recorded in male horses (35.04%; χ^2=13.2653; OR=0.512) in comparison to females (56.48%). No association was found between prevalence of disease and body condition of horse (P=0.2434).

Table 1: Multivariate logistic regression analysis of associated factors with risk of Eimeria infection in horse

Term	Odd's ratio	CI	P Value
Month	1.156	1.088-1.228	0.000
Age	0.422	0.279-0.637	0.000
Sex	0.512	0.325-0.805	0.004
Floor	0.487	0.315-0.755	0.001
Farming type	2.671	1.759-4.057	0.000
Feeding system	1.741	1.123-2.700	0.013
Body condition	0.482	0.314-0.741	0.001

Higher Odds of finding oocysts was recorded in horses kept singly (58.06%; χ^2=11.8863; OR=2.671) in comparison to horses raised in mixed species farming system (42.37%) (Table 1; Fig. 3). Horses fed in troughs (42.61%) were found to be less susceptible to disease in comparison to those given feed on ground (62.17%; χ^2=9.5973; OR=1.741) (Table 1; Fig. 3). Floor type was found to be strongly associated with disease in horses. Higher prevalence was found in horses raised at non-cemented floor (58.04%) in comparison to partially cemented / cemented floor type (41.92%, χ^2=12.5122, OR=0.487) (Table 1; Fig. 3).

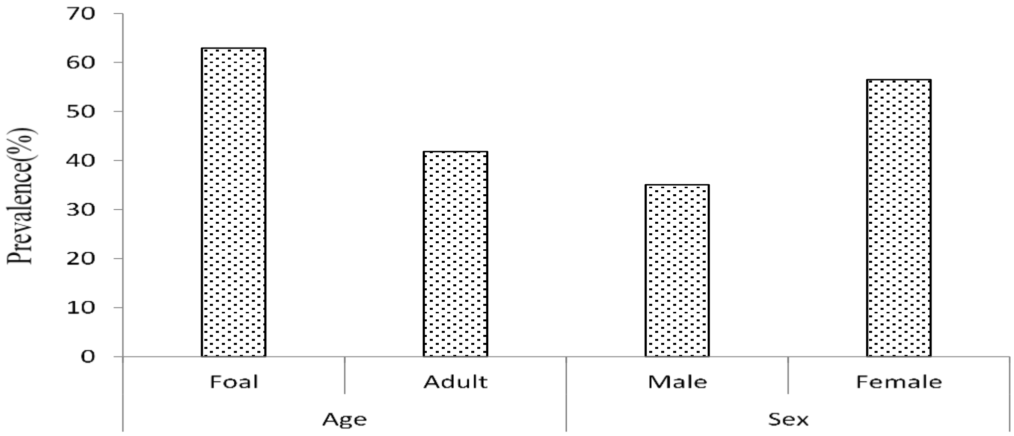

Fig. 2: Prevalence of *Eimeria* in horses in relation to age and sex in the Toba Tek Singh district, Pakistan.

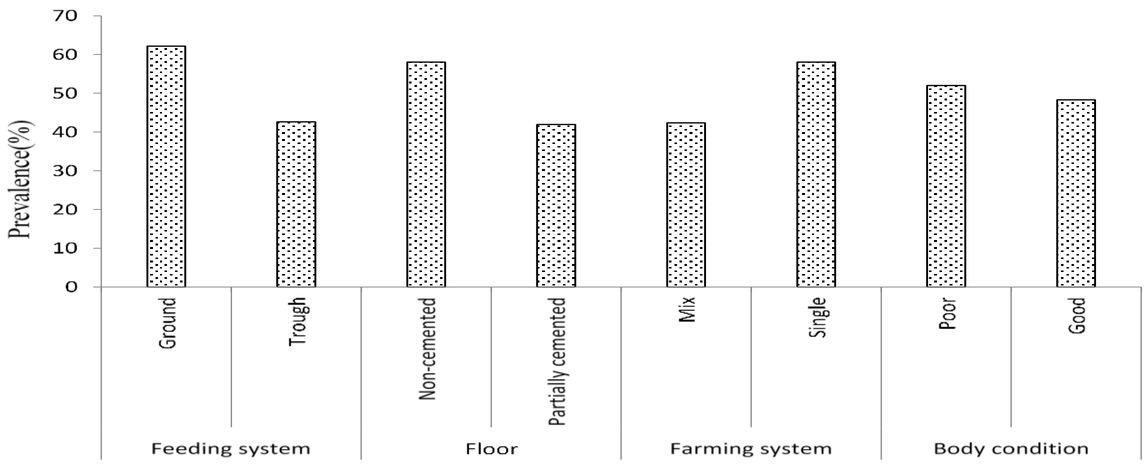

Fig. 3: Prevalence of *Eimeria* in horses for various feeding, floor and farming systems as well as body condition of the animal in the Toba Tek Singh district, Pakistan.

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4. DISCUSSION

Results of present survey first time depicted association of various determinants with the incidence of coccidiosis in horses at large scale. There are various reports of *E. leuckarti* from different parts of world showing prevalence from 0.3 to 80% (Colombia 0.3%, Kashmir 0.34%, Nigeria 1.2%, Romania 1.90%, Greece 4%, Turkey 4.5% & 5.88%, Finland 1% & 5.8%, Poland 6.7%, Iran 7.68%, Nigeria 28.57%, Brazil 40.4%, USA 41.6%, Germany 80%) (Beelitz et al. 1994; Lyons and Tolliver 2004; Studzińska et al. 2008; Pandit et al. 2008; Papazahariadou et al. 2009; Umur and Açici 2009; Ehizibolo et al. 2012; Ionită et al. 2013; Aromaa et al. 2018; Ola-Fadunsin et al. 2019; Ramírez-Hernández et al. 2019; Hautala et al. 2019; Gomes et al. 2019; Khamesipour et al. 2021). Wide range of percentage may be attributed to different techniques of fecal examination and age of sampled animals (Studzińska et al. 2008). Coccidiosis in horses of Europe due to other coccidian have been reported by Gutiérrez-Expósito et al. (2017).

Coccidiosis in horses is believed to be mainly concern of young ones. Ionită et al. (2013) compared prevalence of *E. leuckarti* in foal and adult Romanian horses and found 15% of foals positive in comparison to 1.9% overall prevalence. Ghahfarrokhi et al. (2014) recorded similar results. Ola-Fadunsin et al. (2019) observed an increasing pattern of incidence of disease with increasing age. Dubey and Bauer (2018) reviewed epidemiology of coccidiosis in horses and found that prevalence is higher in foals in comparison to adult horses in all studies. Complex hormonal profile and more contact with young ones may be reason of greater prevalence in females. Ola-Fadunsin et al. (2019) observed higher prevalence of coccidiosis in mares than males. Highest prevalence was observed during months of highest precipitation. Dolenc (1966) reported highest prevalence during months of October, March and April.

It is inferred from findings of this survey that disease can be controlled by improving management systems. Less prevalence in mix farming in comparison to single species farming may be due to fact that *Eimeria* species are host specific. So other species dilute oocyst concentration which resulted in lowered prevalence. Higher prevalence of *E. leuckarti* in animals fed on ground is in line with recommendations of Radostits et al. (2009) to avoid ground feeding as it enhances the chances of contamination of feed with oocysts. Papazahariadou et al. (2009) reported lower parasitic infections in stabled horses as compared to grazing horses. Reduced prevalence was reported by Mitchell et al. (2012) in cattle farms where watering utensil are emptied and cleaned frequently. Aromaa et al. (2018) reported lower prevalence of Eimeria in horse sheds that were cleaned daily. Easy cleaning of cemented floor may be reasoned for less prevalence in animals kept at cemented floor. Also cemented floor dries earlier and low moisture contents results in death of oocysts.

Conclusion: Coccidiosis in horses can be prevented by improving hygienic conditions. Coccidiosis in horses can be prevented by adopting cemented floor, cleaning of farms and hygienic feeding system. Foals and mares should be given special consideration especially during pregnancy.

Author's Contribution: TR conceived and designed experiments. TR performed the experiments. TR, MAZ and AS performed statistical analyses of experimental data. TR prepared the draft of the manuscript. ZS and HMA edited the manuscript. All authors critically revised the manuscript and approved the final version.

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