



Short Communication

Epidemiology and Early Detection of Goat Grub, *Przhevalskiana silenus*, in Northern Mountainous Region of Balochistan, Pakistan

Saadullah Jan¹, Mohammad Lateef², Ferhat Abbas¹, Azhar Maqbool², Makhdoom A. Jabbar², Hamdullah³, Abdul Razzaq⁴ and Essa Kakar³

¹Centre for Advanced Studies in Vaccinology and Biotechnology (CASVAB), University of Balochistan, Brewery Road, Quetta, Pakistan

²University of Veterinary and Animal Sciences, Outfall Road, Lahore, Punjab, Pakistan

³Livestock and Dairy Development Department, Spiny Road, Quetta, Pakistan

⁴Arid Zone Research Centre, Pakistan Agriculture Research Council, Western Bypass, Quetta, Pakistan

*For correspondence: saadcasvab@gmail.com

Abstract

Studies were conducted on epidemiology and early detection of goat grubs, *Przhevalskiana silenus*, in district Pishin, a mountainous northern region of Balochistan, Pakistan, during April 2011 to March 2012. A total of 2880 goats (240 animals per month) of different age, sex and breed were examined on monthly bases by clinico-parasitological examination. The mean prevalence was 16.59%, while month-wise study showed the highest prevalence during January and lowest during October. Age-wise study showed highest prevalence in the age group of 1-2 years as compared to the age groups of < 1 year and > 2 years. However, there was no ($P < 0.05$) difference between males and females, and the two breeds included in the study. For early detection of the infestation, a total of 480 blood samples were collected from the field animals on monthly basis during the same study period. The anti-hypoderma antibodies were detected by ELISA in the sera samples of May and June (summer) onward, while the infestation is usually detected clinically by manual palpation method from October until February (winter) when warbles appear on the back and flank region of the animals. It is concluded that the best time for treatment of goat warble fly infestation in northern mountainous region of Balochistan, Pakistan, is in the months of June and July when first larval instars are still in migratory stage and have not yet caused damage to the skin. © 2014 Friends Science Publishers

Keywords: Epidemiology; Early detection; ELISA; *Przhevalskiana silenus*; Mountainous region; Balochistan; Pakistan

Introduction

Heavy economic losses in livestock sector have been reported due to parasitic diseases in Balochistan (Shafiq and Kakar, 2006). Among these, hypodermosis is also a common infestation and endemic in cattle and goats in Pakistan (Khan *et al.*, 1994, 2006; Babar *et al.*, 2012). Goat grub, *Przhevalskiana silenus*, belongs to order diptera family oestridae, is economically a very important fly whose larvae are obligatory parasites of their hosts and cause severe damage to the skins and hides and ultimately effecting leather industry. The livestock in Pakistan produces 36.3 million numbers of skins and 7.5 million numbers of hides annually, which contribute as important export items of livestock industry and its share is 12% of the total export (Anonymous, 2003). Export of leather and leather goods are one of the largest sources of foreign exchange for Pakistan (Anonymous, 2002). The warble fly infested skin devalues up to 70% depending upon the intensity (Anonymous, 2008), which is the most important

constraint in uplifting the market of hides and skins (Shafiq and Kakar, 2006; Shahid *et al.*, 2013). In the past, detection of goat grub was entirely relied on palpation of the warbles in the back and flank region of the animals or visual examination of the carcasses in the slaughterhouses. By this method, the infections were usually underestimated and neglected. However sensitive and reliable immunological techniques such as enzyme linked immunosorbant assay (ELISA) have solved this problem and can detect the infected animal accurately (Sinclair and Wassall, 1983). ELISA has been used in different countries including Britain to monitor the warble fly eradication program (Tarry, 1986). Keeping in view the importance of this disease in goats, the present study on early detection of goat grub "*Przhevalskiana silenus*" was undertaken in district Pishin, a mountainous region of Balochistan, Pakistan. It is expected that the result of this study will be helpful to plan better control and eradication measures of warble fly in order to reduce the huge economic losses to the leather industry of the country.

Materials and Methods

Studies were conducted on epidemiology and early detection of goat grubs, *Przhevalskiana silenus*, in district Pishin, a northern mountainous region of Balochistan, Pakistan during April 2011 to March 2012.

Profile of Study Area

District Pishin lies between 30° 04' to 31° 17' North latitude and 66° 13' to 67° 50' East longitudes. The area of the district is 5,850 sq km, which is bounded by Qilla Abdullah in north, Qilla Saifullah in east, Quetta and Ziarat in the south and Afghanistan in the west. The general characteristic of the district is mountainous. The mountains are fairly uniform with long central ridges from which frequent spurs descend, which varies in elevation from 1500 to 3300 m. The climate is generally dry and on the whole temperate with very cold and minimum temperature (20.5 to -1.7°C), while summer is relatively mild (30.6 to 35.9°C). The district lies outside the range of monsoon currents. The rainfall is scanty and irregular with an annual average rainfall of 308.2 mm (Anonymous, 1997).

Experimental Design

For epidemiological study, a total of 2880 goats (240 per month) of different age, sex and breed were examined in the field by clinico-parasitological method in different areas of Pishin district, a northern upland region of the province, on monthly bases. Three age groups of the goats i.e. < 1 year, 1-2 year and > 2 year old were selected for the study. Treatment of infection is necessary before the appearance of warbles on the back of animals, which causes severe economic losses. For this purpose early detection of the infection was performed serologically by applying commercial ELISA kit (Otranto *et al.*, 1999, 2005; Faliero *et al.*, 2001). A total of 480 blood samples (40 samples per month) of goats were collected randomly on monthly basis throughout the year. Sera were separated after the blood samples have been clotted and centrifuged where necessary (Cencek and Ziomko, 2002). The sera were stored at -20°C until further use (Boulard and Villejoubert, 1991; Otranto *et al.*, 1999; Ahmed *et al.*, 2011).

ELISA Test Procedure

The test procedure was followed according to the manufacturer of the kit (IDEXX hypodermosis serum antibody test). Briefly, dispensed 190 µL of dilution buffer N. 2 into each well of the 96 well microtitration plate already coated with first instars larvae (L1) of *Hypoderma lineatum*. Undiluted 10 µL of negative control was dispensed in two wells (A1 and A2) and 10 µL of undiluted positive control was dispensed in other two wells (A3 and

A4) of the plate. 10 µL of undiluted sera samples were then added into all remaining 92 wells of the plate. The microtitration plate was covered with a lid and incubated at 37°C in a microplate shaker incubator for one hour. The plate was washed three times with approximately 300 µL of washing buffer (1: 20 with distilled water) and the liquid contents were discarded after each wash. 100 µL of diluted conjugates (1: 100 in dilution buffer No. 1) was dispensed in each well. Microtitration plate was covered with another plate and incubated for half an hour at 37°C. After incubation the plate was washed three times with approximately 300 µL of washing buffer and the liquid contents were aspirated. In each well of the plate 100 µL of TMB substrate (N. 13) was added and incubated for 20 minutes at 18-26°C in a dark place. 100 µL of stop solution (N. 3) was then added in each well of the plate and shaken gently by tapping. The optical density values of samples and controls were measured at 450 nm and the results were calculated for seropositivity.

Climatic Factors

The information of climatic factors regarding temperature, relative humidity and rainfall was collected from Land and water Research program, arid zone research centre (AZRC), Brewery road Quetta. These climatic factors were correlated with the warble fly infestation in goats.

Statistical Analysis

To determine the significance of difference among different variables like month, age, sex and breed, the Pearson Chi-square (χ^2) test was applied (Otranto and Puccini, 2000; Khan *et al.*, 2006; Panadero *et al.*, 2010) by using SPSS, version 16.

Results

Based on the clinico-parasitological examination, the highest (51.25%) prevalence was observed during January 2012 and the lowest (24.58%) during October 2011, while no animal was found infested from April to September 2011 and March 2012 (Fig. 1); whereas, the mean prevalence was 16.59% \pm 21.35. Significant differences ($P < 0.05$) were observed in the prevalence among different age groups. The prevalence was 11.56, 24.89 and 12.39% in age groups of < 1 year, 1-2 year and > 2 years, respectively (Fig. 2). There was no difference ($P < 0.05$) in prevalence between male (12.77%) and females (14.23%) (Fig. 3), and the two breeds being 15 and 13.68% in Khurasani (K) and Non-Descriptive (N) breeds, respectively (Fig. 4).

For early detection of the natural infestation in goats, sera samples were collected monthly throughout the study period and processed by ELISA. The infestation was first detected in the sera samples of May and June, summer season (Fig. 5); whereas, the natural infestation was usually

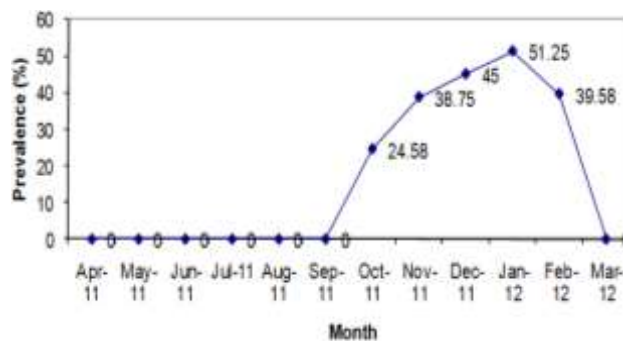


Fig. 1: Month-wise prevalence of goat hypodermosis in district Pishin

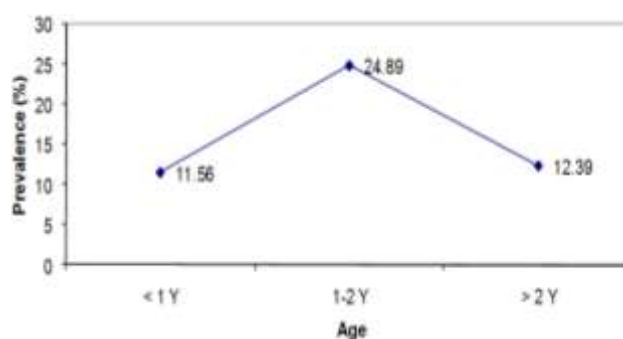


Fig. 2: Age-wise prevalence of goat hypodermosis in district Pishin

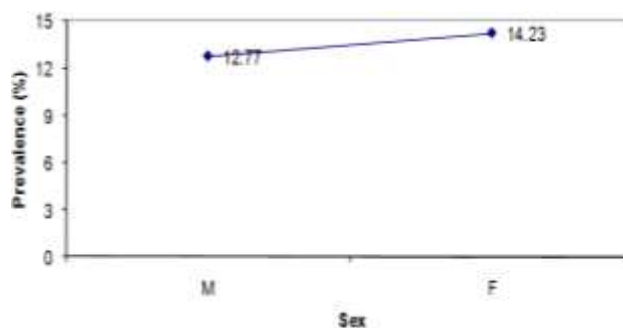


Fig. 3: Sex-wise prevalence of goat hypodermosis in district Pishin

detected clinically in the months of October until February (winter) when warbles appear on the backs of the animals.

Discussion

Prevalence of hypodermosis has been reported with a large variation among different months of the year, sex of animals and their breeds. As recorded in the current study, by and large, prevalence has been reported higher in colder compared with warmer months elsewhere (Khan *et al.*, 2006; Anonymous, 2008). Prevalence in young animals has been reported higher compared with the older animals.

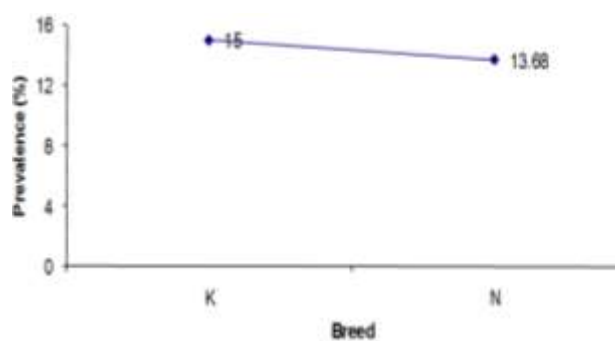


Fig. 4: Breed-wise prevalence of goat hypodermosis in district Pishin

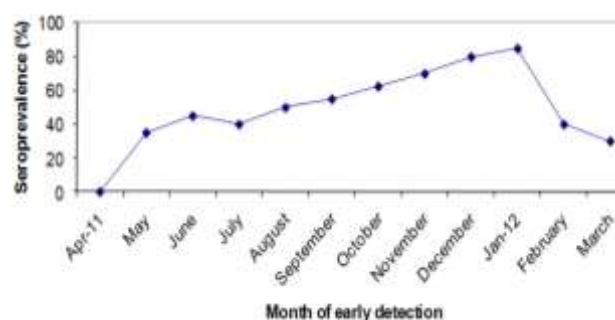


Fig. 5: Early detection of goat hypodermosis by ELISA in district Pishin

These findings support the earlier reports from different regions of the world (Murray, 1967; Robertson, 1980; Oryan *et al.*, 2009; Hassan *et al.*, 2010). Low prevalence of hypodermosis in older animals may be attributed to acquired resistance in older animals after two or three repeated infestations (Tassi *et al.*, 1989; Perez *et al.*, 1995; Oryan *et al.*, 2009; Hassan *et al.*, 2010). Lower prevalence in older age group may also be due to thickness and hardness of the skin from which the larvae cannot penetrate easily as compared to the younger age group (Oryan *et al.*, 2009). In another study conducted by Asbakk *et al.* (2005), level as 10% of antihypoderma antibody in adults of reindeer declined significantly with age, and levels were significantly lower in animals of 4 to 11 years of age than in one year old animals during the same one year period indicating reducing exposure of animals to hypoderma infestation. Results of the present study did not reveal any association of sex or breed of the animal with prevalence of hypoderma, which support the earlier findings (Perez *et al.*, 1995; Rahbari and Ghasemi, 1997; Oryan *et al.*, 2009).

Warble fly larvae are endoparasitic for most of the year and the use of serological method (ELISA) increases the period of diagnosis. Therefore, an early detection and early treatment of infestation is necessary before the appearance of warbles on the back of animals to cause economic losses (Jackson *et al.*, 1998). In the present study, serological study for an early diagnosis of hypodermosis

was carried out in which the warble fly infestation was detected by ELISA as early in the sera samples of May and June (summer), while the infestation is usually detected clinically in the months of October until February (winter) after observing the warbles on the back and flank regions of the animals. Antibodies against hypoderma have been detected as early as 6 weeks post infestation in artificially infested steers by Colwell and Baron (1990). Otranto and Puccini (1998) have reported that October to December were the best time for obtaining samples and treating the animals while *P. silenus* larvae were still in migratory phase and had not yet caused any damage to the skin. Faliero et al. (2001) stated that the highest goat warble fly infestation in November and December proved to be the optimal period for blood sampling to perform an early diagnosis. In an artificial infestation of previously uninfected group, antibodies were first detected in 7th week post infestation, rose to maximum level between 24th and 25th weeks and then declined as larvae reached the back of the animals while in previously exposed groups antibodies were first detected in 3rd week post infestation with peak antibody levels in 8th Week (Colwell et al., 2008). The farmers usually treat their animals against warble fly in winter season when they observe the warbles on the back of the animals but at this stage the larvae have had damaged the skins of the animals. It is therefore concluded and recommended that the best time for the treatment of goat warble fly infestation in uplands of Balochistan is in the months of June and July when first larval instars (L1) are still in migratory stage and not yet have reached their final sites on the back of goats.

References

- Ahmed, H., B. Mirza, Q. Khan, W. Safdar and M. Qayyum, 2011. Seroprevalance of hypodermosis (*Hypoderma lineatum*) in the cattle of potohar region, Pakistan. *Int. J. Cell. Mol. Biol.*, 2: 497–503
- Anonymous, 1997. *Pishin-A District Profile*, pp: 3–7. Govt. of Balochistan, Bureau of Statistic, Planning Studies Section, Planning and Development Dept., Quetta, Pakistan
- Anonymous, 2002. *Economic Survey of Pakistan*. Economic Advisor Wing, Finance Division, Islamabad, Pakistan
- Anonymous, 2003. *About Pakistan, Cyber Wing*. Ministry of Information and Broadcasting, Government of Pakistan
- Anonymous, 2008. *Annual Report*. National Agriculture Research Center, Government of Pakistan, Islamabad, Pakistan
- Asbakk, K., A. Oksanen, M. Nieminen, R.E. Haugerud and A.C. Nilssen, 2005. Dynamics of antibodies against hypodermin C in reindeer infested with the reindeer warble fly *Hypoderma tarandi*. *Vet. Parasitol.*, 129: 323–332
- Babar, W., Z. Iqbal, M.N. Khan and G. Muhammad, 2012. An inventory of the plants used for parasitic ailments of animals. *Pak. Vet. J.*, 32: 183–187
- Boulard, C. and C. Villejoubert, 1991. Use of pooled serum or milk samples for the epidemiological surveillance of bovine hypodermosis. *Vet. Parasitol.*, 39: 171–183
- Cencek, T. and I. Ziomko, 2002. Optimal time for serologic diagnosis of hypodermosis in different regions of Poland. *Bull. Vet. Inst. Pulawy*, 46: 59–63
- Colwell, D.D. and R.W. Baron, 1990. Early detection of cattle grub (*Hypoderma lineatum* and *H. bovis*) (Diptera, Oestridae) using ELISA. *Med. Vet. Entomol.*, 4: 35–42
- Colwell, D.D., C. López, P. Diez-Baños, P. Morondo and R. Panadero, 2008. Impact of previous infestation on dynamics of circulating hypodermin C in cattle artificially infested with *Hypoderma lineatum* (Diptera: Oestridae). *Vet. Parasitol.*, 154: 114–121
- Faliero, S.M., D. Otranto, D. Traversa, A. Giangaspero, G. Santagada, R. Lia and V. Puccini, 2001. Goat warble fly infestation by *Przhevalskiana silenus* (Diptera: Oestridae): immunoepidemiologic survey in the Basilicata region (southern Italy). *Parassitologia*, 43: 131–134
- Hassan, M., M.N. Khan, M. Abubakar, H.M. Waheed, Z. Iqbal and M. Hussain, 2010. Bovine hypodermosis—a global aspect. *Trop. Anim. Health Prod.*, 42: 1615–1625
- Jackson, F., M.A. Taylor and D.E. Jacobs, 1998. Diagnosis of Animal Parasitism. *Parasitol. Today*, 14: 295–297
- Khan, M.Q., S. Akhtar and A.H. Cheema, 1994. Efficacy of Ivermectin against Goat warbles in Pakistan. *Vet. Rec.*, 135: 361
- Khan, M.N., Z. Iqbal, M.S. Sajid, M. Anwar, G.R. Needham and M. Hassan, 2006. Bovine hypodermosis: Prevalence and economic significance in southern Punjab, Pakistan. *Vet. Parasitol.*, 141: 386–390
- Murray, V.I.E., 1967. Studies on *hypoderma lineatum* de villiers and *hypoderma bovis* degeer (diptera, hypodermatidae) in manitoba. *Can. Vet. J.*, 8: 17–22
- Oryan, A., S.M. Razavi and S. Bahrami, 2009. Occurrence and biology of goat warble fly infestation by *Przhevalskiana silenus* (Diptera, Oestridae) in Iran. *Vet. Parasitol.*, 166: 178–181
- Otranto, D., V. Puccini, P. Freccia and A. Giangaspero, 1998. Goat warble fly infestation (*Przhevalskiana silenus*). The use of the kit-ELISA Hypodermosis for serological diagnosis on milk and blood sera. *O and DV Obiettivi e Doc. Vet.*, 19: 51–56
- Otranto, D., C. Boulard, A. Giangaspero, M.P. Caringella, D. Rimmele and V. Puccini, 1999. Serodiagnosis of goat warble fly infestation by *Przhevalskiana silenus* with a commercial ELISA kit. *Vet. Rec.*, 144: 726–729
- Otranto, D. and V. Puccini, 2000. Further evidence on the internal life cycle of *Przhevalskiana silenus* (Diptera, Oestridae). *Vet. Parasitol.*, 88: 321–328
- Otranto, D., P. Zallab, G. Testinia and S. Zanajc, 2005. Cattle grub infestation by *Hypoderma* spp. in Albania and risks for European countries. *Vet. Parasitol.*, 128: 157–162
- Panadero, R., C. Martí nez-Carrasco, L. Leo n-Vizcai no, C. Lo p ez, P. Di e z-Ban os, M.P. Morondo and F. Alonso, 2010. Use of a crude extract or purified antigen from first-instar cattle grubs, *Hypoderma lineatum*, for the detection of anti-*Hypoderma* antibodies in free-ranging cervids from southern Spain. *Med. Vet. Entomol.*, 24: 418–424
- Perez, J.M., J.E. Granados and I. Ruiz-Martí nez, 1995. Studies on the hypodermosis affecting red deer in central and southern Spain. *J. Wildlife Dis.*, 31: 486–490
- Tassi, P., V. Puccini and A. Giangaspero, 1989. Infection by the warble fly *Przhevalskiana silenus* Brauer, 1858, in the Italian goats. An update up to 1988. *Parassitologia*, 31: 239–250
- Rahbari, S. and J. Ghasemi, 1997. Study on economic aspects of goat grubs in Iran. *Trop. Anim. Health Prod.*, 4: 243–244
- Robertson, R.H., 1980. Antibody production in cattle infected with *Hypoderma* spp. *Can. J. Zool.*, 58: 245–251
- Shafiq, M. and M.A. Kakar, 2006. Current livestock marketing and its future prospects for the economic development of Balochistan, Pakistan. *Int. J. Agric. Biol.*, 8: 885–895
- Shahid, M., S. Janjua, F.I. Abbas and J.S. Burbach, 2013. PCR-based detection of *Trypanosoma evansi* infection in semi-captive asiatic black bears (*Ursus thibetanus*). *Pak. Vet. J.*, 33: 442–445
- Sinclair, I.J. and D.A. Wassall, 1983. Enzyme-linked immunosorbent assay for the detection of antibodies to *Hypoderma bovis* in cattle. *Res. Vet. Sci.*, 34: 251–252
- Tarry, D.W., 1986. Progress in warble fly eradication. *Parasitol. Today*, 2: 111–116

(Received 25 April 2013; Accepted 28 May 2013)