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Sero-epidemiological Studies on Goat Hypodermosis in Northern Upland Balochistan, Pakistan

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Abstract.- Sero-epidemiological studies were conducted on goat warble fly, *Przhevalskiana silenus,* to observe the prevalence of hypodermosis in district Ziarat, northern upland of Balochistan, Pakistan during April 2011 to March 2012. The most prevalent breed of goat 'khurasani' was involved in the study and compared with the non-descript breed of goat. In the first study a total of 2880 (240 animals per month) goats of different age, sex and breed were examined on monthly bases. The mean percentage was 21.25% with the highest prevalence in January and lowest in October. Three age groups were selected for the study *i.e.* < 1year, 1-2 year and > 2 year old in which the highest percentage was noted in 1-2 years of age group. No significant differences were observed between both sexes and both breeds. In the second study, sero-prevalence was studied by commercial ELISA kit. A total of 480 sera samples were collected during April 2011 to March 2012. Three hundred and eight (64.16%) samples were found sero-positive. Data analyzed by Pearson chi-square test ($\chi 2$) revealed significant difference ($P< 0.05$) month-wise in the prevalence studies. It is concluded that the commercial ELISA kit is a useful tool for the serodiagnosis of goat warble fly infestation which provides a base line data for the control and future eradication program of this economically important parasite.

Keywords: Sero-epidemiology, goat hypodermosis, ELISA, upland Balochistan.

INTRODUCTION

Among the common ectoparasites of goats, warble fly, *Przhevalskiana silenus,* commonly exists in Pakistan (Shah *et al.*, 1981; Khan *et al*., 1991; Ayaz, 1998) which belongs to order Diptera family Oestridae. An adult female lays 100 eggs (Sayin *et al*., 1973) from which first instar larvae (L1) hatch in 5 to 6 days and penetrate into the skin by means of paired mouth hooks and collagenase enzymes, hypodermine C (Madel and Nahif, 1971). First instar larvae (L1, May to October) moult to second instar (L2, October to December) and then to third instar (L3, January to March) in the subcutaneous tissues of the back and flank region of the goats (Otranto and Puccini, 2000), drop on the ground and pupate from which adult flies emerge (Tassi *et al*., 1989). It is economically a very important fly whose larvae are obligatory parasites of their hosts and cause severe damage to the skins affecting leather industry. This infection was first reported in Italy (Brauer, 1858).

In the province of Balochistan 11.8 million of goats are raised (GOP, 2006). Khurasani is one of the major breeds of goat mainly raised for mutton and hair (Anonymous, 2002b; Munir *et al*., 2010). The northern uplands of Balochistan including Khurasan, Toba Kakari, Suleiman mountains region of Zhob and Sherani districts, Qilla Saifullah, Loralai, Ziarat and Pishin districts are the main habitats of the Khurasani breed. Phenotypically this breed is having long black hair coat and turned back horns. The breed is predominantly black in color with red face but some other color may be found occasionally. The animal is smaller in size and good in milk production (0.9-1.3L/day). The hair is used for ropes, rugs and tents (www.pastoralpeoples.org/

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docs/CGRFA_LIFE_sideevent_kakar.pdf.). Skins and wool from small ruminants provide raw material for the leather and wool-garment industries, respectively. The traditional woolen blankets and rugs are major source of extra income for families dependent on livestock in Balochistan (Anonymous, 2002b).

The husbandry practices play important role in the exposure of the parasite. Animals in uplands of Balochistan are mostly dependent on open grazing system, one of the most important factors for warble fly infestation (Otranto *et al*., 2005), thus continuously exposed to warble fly infestation. Kidding period in uplands is from March to April while the adult fly activity season starts after mid April. Kids are mostly not exposed to grazing because they are usually kept in door for a few months from birth to weaning age. They come in contact with their dams during night when their dams come back after grazing at sun set.

Goat warble fly infestation has severe economic impact on tanning industries, impaired milk and meat production, growth retardation and also carcass depreciation (Oryan *et al*., 2009). The livestock in Pakistan produces 36.3 million numbers of skins and 7.5 million numbers of hides annually which are important export items of livestock industry that contribute 12 percent of the total export (Anonymous, 2003). Export of leather and leather products are Pakistan's one of the largest source of foreign exchange (Anonymous, 2002a). The warble fly infested skin reduces the prices up to 70% depending upon the numbers of holes formed by the larvae of warble fly (Anonymous, 2008). The poor quality of hide and skin is the most important constraint affecting the marketing of hides and skins which is due to warble fly infestation along with other factors including poor flaying techniques and improper preservation (Shafiq and Kakar, 2006).

The diagnosis of warble fly infestation in the past was entirely relied on palpation of the warbles in the backs of the animals or visual examination of the carcasses in the slaughterhouses in which 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, Sinclair *et al*., 1984). Serological studies were conducted by commercial ELISA kit (Otranto *et al*., 1999, 2005; Faliero *et al*., 2001) which was basically developed for bovine hypodermosis (IDEXX hypodermosis serum antibody test) but has also been validated for use in goats, deer, camel and men (Morsy *et al*., 1999; Domínguez *et al*., 2010; Panadero *et al*., 2010; Puente *et al*., 2010).

Although several studies have been conducted on epidemiology, taxonomy, biology, histopathology, economic losses, and immunology of warble fly infestation in bovines, very little attention has been given to caprine hypodermosis in Pakistan, particularly in the province of Balochistan. Keeping in view the importance of warble fly infestation in livestock, the present study was undertaken in northern upland of Balochistan, Pakistan. It is expected that the result of this study will be helpful providing the base line data to plan better control and eradication measures of warble fly for further improvement in the livestock sector of Balochistan, Pakistan.

MATERIALS AND METHODS

Studies were conducted on sero-epidemiology of warble fly infestation in goats in district Ziarat, northern uplands of Balochistan, Pakistan. The duration of the study was one year commenced from April 2011 to March 2012.

Profile of the study area

District Ziarat is located at 30° 22' 47 N 67° 43' 38 E with an altitude of 2543 meters (8346 ft). It comprised an area of 1489 km^2, with climate mild in summer and freezing cold in winter. The area is refreshingly cool in summer, from May to August. It starts getting cold in September and is extremely cold during November to March. In winter, it gets a good amount of snowfall. Average rainfall is 20.60 mm. The months of January to March in winter and July and August in summer receive most of the rainfall. Ziarat is also very famous for its juniper forest. Juniper species *Juniperous macropoda*, *Juniperous exceisa polycarpus* or *Pashthani juniper* are found in Ziarat districts and Zarghoon mount and some other mounted areas of Balochistan

known as the second largest juniper forest in the world, which covers an area of about 247,000 acres (1,000 km^2). The other major species are wild ash, wild almond, *Olea* species, Khujak and *Ephedra sinica*. *Artimisia maritime, Cymbopogon, Crysopogoneri* (Anonymous, 1997b).

Table I.- Distribution of goats according to breed, age and sex for warble fly examination.

Breed	Age (Years)	Sex		Total
		Male	Female	
Khurasani	< 1 Year (40/monthx12)	240 (20/Month x 12)	240 (20/Month x 12)	480
	1-2 Year (40/monthx12)	240 (20/Month x 12)	240 (20/Month x 12)	480
	>2 Year (40/monthx12)	240 (20/Month x 12)	240 (20/Month x 12)	480
Non descript	< 1 Year (40/monthx12)	240 (20/Month x 12)	240 (20/Month x 12)	480
	1-2 Year (40/monthx12)	240 (20/Month x 12)	240 (20/Month x 12)	480
	>2 Year (40/monthx12)	240 (20/Month x 12)	240 (20/Month x 12)	480
Total		**1440**	**1440**	**2880**

Experimental design

In the first study, a total of 2880 goats of different age group (< 1year, 1-2 year and > 2 year), sex and breed were examined by hand palpation method using cluster sampling technique (Thrusfield, 2007). The detail is given in Table I. To meet the required sample size fulfilling the criteria of age, sex and breed, several flocks of different sizes (ranged from 50 to 250 heads per flock) were selected from the whole population. Age was determined by incisor teeth in the lower jaw (Khan, 1969). Breed identification was accomplished from their phenotypic characteristics (Hasnain, 1985). Second study was conducted on month-wise sero-prevalence by commercial ELISA kit method (Otranto *et al*., 1999, 2005; Faliero *et al*., 2001). A total of 480 blood samples (40 samples per month, regardless of breed, age and sex) were taken randomly. Blood sample were not concerned whether the goat is having lesions or not but were also included in the prevalence study to correlate the two methods. The animals selected for blood sampling were neither re-sampled monthly nor physically examined for the lesions. 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

The test was performed according to the protocol of manufacturer of the kit (IDEXX hypodermosis serum antibody test).

Statistical analysis

To determine the significance of difference in prevalence among different variables like month, age, sex and breed, Pearson chi-square ($\chi 2$) test was applied (Otranto and Puccini, 2000, Khan *et al*., 2006; Azizi *et al*., 2007; Panadero, 2010) by SPSS, version 16.

RESULTS

Month, breed, age and sex-wise prevalence of goat hypodermosis was studied in district Ziarat during April 2011 to March 2012 (Table II). The prevalence of palpable lesions was highly seasonal, as lesions were detected only between October 2011 and February 2012. The highest prevalence of goats with palpable lesions was 66% in January 2012. There were no significant effects of breed or sex but the prevalence was significantly higher in 1-2 years old goats than in either those < 1 year- or > 2 years old. In the second study, significant difference was observed in sero-prevalence among the months with the highest (92%) value during November and lowest (20%) during March (Table III) with a mean of 64.12%. Although there were no obvious clinical palpable lesions from March to September in the palpation method, the larvae at that time were at subclinical stage (L1) which were detected by serological test (ELISA) to compare the serological (sub-clinical) and palpation (clinical) methods regarding early detection of the infestation.

Month-wise analysis of data revealed significant differences ($P<0.05$) both in the prevalence and sero-prevalence (regardless of breed,

Table II.- Month, breed, age and sex-wise prevalence of goat hypodermosis in district Ziarat during April 2011 to March 2012.

Month	Breed		Age (Years)			Sex		Month-wise percentage (%)	*Mean Ranks
	Khurasani	Non-descript	<1	1-2	> 2	Male	Female		
April 2011	0.0	0.0	0	0	0	0.0	0.0	0.0	1134.50^{e}
May	0.0	0.0	0	0	0	0.0	0.0	0.0	1134.50^{e}
June	0.0	0.0	0	0	0	0.0	0.0	0.0	1134.50^{e}
July	0.0	0.0	0	0	0	0.0	0.0	0.0	1134.50^{e}
August	0.0	0.0	0	0	0	0.0	0.0	0.0	1134.50^{e}
September	0.0	0.0	0	0	0	0.0	0.0	0.0	1134.50^{e}
October	37.5	35.0	22.5	61.25	25	36.7	35.8	36.25	1656.50^{d}
November	49.2	43.3	32.5	71.25	35	49.2	43.3	46.25	1800.50^{c}
December	52.5	48.3	37.5	77.5	36.25	49.2	51.7	50.41	1860.50^{bc}
January 2012	70.0	61.7	53.75	88.75	55	65.8	65.8	65.83	2082.50^{a}
February	61.7	50.8	46.25	80	42.5	55.8	56.7	56.25	1944.50^{b}
March	0.0	0.0	0	0	0	0.0	0.0	0.0	1134.50^{e}
Total	**22.6**	**19.9**	**16.04**	**31.56**	**16.14**	**21.4**	**21.1**	**21.25**	

*Mean ranks with different superscripts (abcde) in each row indicate significant difference (P<0.05) between months.

Table III.- Month-wise seroprevalence of goat hypodermosis in district Ziarat.

Month	Sera tested	Positive	Sero-prevalence (%)	*Mean Ranks
April 2011	40	_	_	87.00^{e}
May	40	23	57.5	225.00^{c}
June	40	26	65	243.00^{bc}
July	40	30	75	267.00^{b}
August	40	32	80	279.00^{ab}
September	40	34	85	291.00^{ab}
October	40	30	75	267.00^{b}
November	40	37	92	309.00^{a}
December	40	28	70	255.00^{b}
January 2012	40	34	85	285.00^{ab}
February	40	26	65	243.00^{bc}
March	40	8	20	135.00^{d}
Total	**480**	**308**	**64.16**	

***Mean ranks with different superscripts (abcde) in each row indicate significant difference (P<0.05) between months.

age and sex) of goat hypodermosis. In the study of palpation method there was also significant difference (P< 0.05) amongst age groups, while no significant difference was observed between both sex and breeds.

DISCUSSION

The sero-epidemiological studies provide the initial and baseline data to plane better strategies for the control and eradication of a disease in the country. The present study on goat hypodermosis was therefore carried out for the first time in district Ziarat, northern upland of Balochistan, Pakistan, to estimate the infestation rate and to diagnose the larvae by ELISA at the earlier sub-clinical stage. There are many factors that contribute in the prevalence of warble fly infestation. Among those one of the important factors include grazing pattern (Otranto *et al*., 2005, Ahmed *et al*., 2013). Animals in the study area are mostly dependant on rangeland grazing, thus continuously exposed to warble fly infestation which agrees with the study conducted in Italy where the seroprevalence was highly correlated with the free grazing practices (Frangipane di Regalbono *et al*., 2003). Month-wise, age-wise, sex-wise and breed-wise prevalence was studied by examining goats in the field. Significant differences were observed in the prevalence among months in one year study. The higher prevalence was observed from November to January which is almost similar to the studies of Fuente-Lopez *et al*. (2001) in which variations in prevalence were observed between seasons, with higher values from November to February, and maximum of 94.4% prevalence in November. The present study also coincides with that of Khan *et al*. (2006) in which highest month-wise prevalence was recorded in December and the lowest in July. The month-wise prevalence under agriculture linkage program (ALP) project in

Balochistan also showed highest prevalence during November and January in various areas of the province (Anonymous, 2008).

Prevalence was determined in three different age groups (< 1year, 1-2 year and > 2 years) of goats. Significant differences were observed in prevalence among these age groups with highest in 1-2 years old goats than < 1year or > 2 years old. This is in agreement with the studies conducted in various regions of the world (Murray, 1967; Robertson, 1980; Yadav *et al*., 2006; Azizi *et al*., 2007; Oryan *et al*., 2009; Hassan *et al*., 2010; Ahmed *et al*., 2013). Adults showed a significantly low infection in the field animals. In cattle and goats hypodermosis, the younger animals are more susceptible than repeatedly exposed older animals and has a higher mean intensity of parasitism than older animals (Tarry, 1987; Puccini *et al*., 1987) because an acquired resistance is established in older cattle after two or three repeated infestations (Boulard, 1987; Oryan *et al*., 2009; Hassan *et al*., 2010, Ahmed *et al*., 2013). These observations were also in agreement with the studies conducted in some other parts of the world (Fuente-Lopez *et al*., 2001). Oryan *et al*. (2009) studied that 25% of the larvae were dead in older goats while in younger goats the percentage of dead larvae was 10%. 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 a study conducted by Asbakk *et al*. (2005), anti-hypoderma antibody level (10%) in adults 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.

In animals less than one year of age, the prevalence was significantly lower than the age group of 1-2 years. Kidding period in the study area is from mid March to mid April (spring season), while hypoderma is in pupal stage at this time and the adult fly activity season (oviposition) is in the mid of April (early summer). The age group less than one year was mostly not exposed to grazing, because the kids are usually kept indoor for a few months from birth to weaning age and until that time the adult fly activity season had finished. This is also supported by other studies (Fuente-Lopez *et al*., 2001; Oryan *et al*., 2009; Otranto *et al*., 2005) in which the prevalence was low in age group of less than one year.

No significant difference was observed in prevalence between either sexes which agrees with the studies conducted in different countries of the world (Perez *et al*., 1995; Rahbari and Ghasemi, 1997; Fuente-Lopez *et al*., 2001; Yadav *et al*., 2006; Oryan *et al*., 2009). Similarly there was no significant difference in the breeds as reported by Yadav *et al*. (2006).

Serological technique such as ELISA has been shown to detect the infected animal accurately and it has been used in many parts of the world for the detection of warble fly infestation. It is simple, rapid, useful tool and a good alternative of clinical palpation method (Panadero *et al*., 2010). Based on ELISA, the warble fly has been eradicated from many northern European countries including Denmark, Netherland, the Czech Republic, Germany, France, Switzerland (Otranto *et al*., 2005), United Kingdom and Ireland (Colwell and Otranto, 2008). The commercial ELISA kit developed for bovine hypodermosis can be considered as a useful tool for the serodiagnosis of goat warble fly infestation due to the confirmed cross-reactivity between the antigen of *Hypoderma lineatum* and anti-*P. silenus* antibody and the simplicity and the rapidity of the assay (Faliero *et al*., 2001; Otranto *et al*., 1997, 2004). Hypodermin C (HyC) is found in several hypoderma species and has been used widely in serological diagnosis of hypodermosis. This enzyme can be used from one species for the serodiagnosis of hypodermosis caused by other species (Asbakk *et al*., 2005). In an experimental infestation technique, cross-transmissional study of *Hypoderma lineatum* from cattle to domestic goats was conducted in which the appearance of antibodies on ELISA suggested that some larvae survived enough to initiate a humoral immune response (Colwell *et al*., 2006). The results of other studies confirmed that the first instar antigen of *H. lineatum* can also be used to diagnose natural infection by *H. actaeon* in deer and *H. sinense* in man (Domínguez *et al*., 2010; Panadero *et al*., 2010; Puente *et al*., 2010). There are also cross reactions between anti-hypoderma antibodies and antibodies against both *Przhevalskiana silenus*

and *Cephalopina titillator* (Morsy *et al*., 1999).

In the present study the overall mean sero-prevalence was 64.16%. The height of district Ziarat is 2543 meters above sea level and this high seropositivity percentage is directly related to the altitude which was also observed by Faliero *et al*. (2001) that in the mountainous areas seropositivity percentage is greater than the semi hilly and plane areas due to free grazing practices (Otranto *et al*., 2005) and more favorable conditions for the development of the larvae into adult flies. The antibody titer was elevated with the migratory phase of first instar larvae (L1). This is also supported by a sero-epidemiological survey which demonstrated that the kinetics of anti-hypoderma antibodies in yaks and cattle were elevated from October to December, coinciding with the migration of L1 and that the serosurveillances and chemical therapy would best be performed in these months in the region (Guan *et al*., 2005). Same observations were also recorded in Italy (Otranto *et al*., 1998) in which the highest antibody titer was recorded from October to November that coincided with the migration of the larvae inside the animal's body. These months were found suitable sampling period for immunodiagnostic and immunoepidemiological studies of goat warble fly infestation. These observations are also in agreement with the study conducted on antigen capture ELISA for the detection of hypoderma antigen (hypodermin C) in which the levels of antigen fell quickly following arrival of grubs at the back and completion of the molt to second instar (Panadero *et al*., 2002). In a study the antibody levels rose after infestation and reached a maximum in November and December, which coincides with the end of migratory phase of *H. tarandi* larva after it has reached its final site under the skin of the back of animal (Asbakk *et al*., 2005). They further stated that the antibody level decreases or stops when L1 moults to L2 and L3 because hypodermin C encoding gene stops functioning and the secretion of hypodermin ceases. In Canada an experimental infestation of cattle with hypoderma spp. was conducted in which the peak of antibody production was observed when the antigen levels were at peak during migration of first instar larvae to the back of animals but as larvae molted to L2, the antigen production ceased and there was a persistence level of antibody (Colwell *et al*., 2008). Faliero *et al*. (2001) and Otranto *et al*. (2004) stated that ELISA test was found to be an easy and economical tool for goat warble fly infestation. The antibodies became lost from the circulation of the infested animals about 14 weeks after the disappearance of the infection (Sinclair *et al*., 1984; Boulard and Villejoubert, 1991). In Iran the occurrence of goat grubs infestation started from July, reached at peak in September and October, and then declined in February (Rahbari and Ghasemi, 1997). It is concluded that the best treatment time for goat hypodermosis in uplands of northern Balochistan is during June to July when the first instar larvae are still in migratory stage and have not yet caused damage to the skin.

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