



RESEARCH ARTICLE

Seroepizootiological investigation on Goat Warble Fly Infestation (*Przhevalskiana silenus*) in Pothwar Plateau, Pakistan

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ABSTRACT

Goat Warble Fly Infestation (GWFI) is also known as subcutaneous myiasis caused by *Przhevalskiana silenus* (Diptera: Oestridae). It is widely distributed in tropical and sub-tropical areas of the world. In goats, WFI is usually detected through conventional procedure which underestimated the infestation. The current study was designed to determine the serodiagnosis of GWFI (through IDEXX Hypodermosis serum antibody test) and also aimed to investigate its seroepizootiological profile in Pothwar region, Pakistan from 2013-14. The results showed that average seropositivity (ELISA kit) of GWFI was 18.5% whereas, it was 11% by using conventional procedure (Palpation method) depicting a significant difference ($p < 0.05$). Higher seropositivity (30.8%) was observed in Jhelum district as compared to Attock district (6%). The L1 larvae were found in September, while nodules start appearing in October to December and last until the end of February. The month wise peaks of optical density (OD) was higher in December which gradually decrease along with the end of winter season. The prevalence of GWFI revealed no significant difference among three host breeds (Jattal, Beetal and Tedy). According to the results, high infestation rate (28%) was observed in young animals of age group < 1 year as compared to old animals (> 2 years). Topographically, hilly areas (33%) provide favourable climatic conditions for the propagating of larval stages. Sex difference showed no significant difference. The seroprevalence varied significantly with respect to age, month, districts and topographical location. The current study proved that serologic diagnosis (commercial ELISA kit) as more sensitive and accurate for timely diagnosis of GWFI than traditional method. The information on the epizootiology of *P. silenus* in goats of Pothwar region would help in devising effective control strategies.

Keywords: GWFI, *Przhevalskiana silenus*, serodiagnosis, seroprevalence, Pothwar Plateau, Pakistan

INTRODUCTION

Livestock is the backbone of the country like Pakistan where majority of the population especially in the rural area depends upon the agriculture sector. There are different factors responsible for low production in the livestock sector. One of them are ectoparasitic infestations throughout the world (Hourrigan, 1979). Among these, myiasis caused by *Hypoderma* species (larvae) is considered as an economically important menace in wild and domesticated ruminants. The definitive herbivore host of this parasite include buffalo, cattle, deer, reindeer, sheep and goat (Scholl, 1993). In Pakistan, warble fly infestation (WFI) is mainly reported from mountainous and semi-hilly parts in large ruminants (Shah, 1981; Hasan *et al.*, 2007) and in goats (Khan, 1997). Despite, the physical impairment; myiasis harm the internal organs

and also affects the host's immunity. In Europe and North America, this infestation was significantly reduced by chemotherapeutic treatments against the first larval stage and adults of the parasitic fly (Boulard, 2002).

Detection of infested goats by clinical examination (hand palpation) generally render the hypodermosis unrecognized (Sinclair *et al.*, 1984). The accurate and rapid diagnosis of etiological agents and characterization of parasites based on epidemiologically useful features are necessary for the control of veterinary parasitic diseases. Throughout the world, many serological assays immunoelectrophoresis, indirect haemagglutination, and gel diffusion have been developed for early diagnosis of WFI (Balkaya *et al.*, 2010).

Indeed, Enzyme Linked Immunosorbent Assay (ELISA) resolved the difficulty of timely detection of infestation prior to the sign of protuberances and is extensively practiced

because of its accuracy (Otranto *et al.*, 2001). In this assay, hypodermal antigen (1st instar) is used for antibodies detection in infested sera (Boulard & Moiré, 2004). It is well recognized in U.K, where hypodermosis was successfully eradicated through the application of ELISA (Webster *et al.*, 1997). The serodiagnosis of WFI by commercial ELISA kit (IDEXX Hypodermosis serum antibody test) (Otranto *et al.*, 1999; Faliero *et al.*, 2001; Otranto *et al.*, 2005), basically developed for bovine hypodermosis, confirmed cross-reactivity between *Hypoderma lineatum* antigen and anti-*P. silenus* antibodies and validated for use in goats (Morsy *et al.*, 1999; Domínguez *et al.*, 2010; Panadero *et al.*, 2010; Puente *et al.*, 2010).

Goats are considered as poor men's cow in Pothwar region as they help to augment income of rural population. Despite the extensive problem caused by warble fly, the information on serodiagnosis and epidemiological profile of GWFI is not well defined in this region. All previous investigations in the study area were based on traditional palpation procedure and therefore unable to diagnose disease at early stages. This necessitated the need of reliable and efficient diagnostic technique for GWFI to minimize economic losses to the livestock sector of Pakistan.

Therefore, present study was designed with following objectives in Pothwar region, Punjab, Pakistan:- (1) Estimation of seroprevalence of *P. silenus* infestation in goats by serologic diagnosis (commercial ELISA kit) and comparison with traditional method (2) To explore potential risk factors for designing eradication platform for the infestation.

MATERIALS AND METHODS

Study Area

The current serological investigation was carried out among goats of Pothwar region of Punjab, Pakistan. This region, comprises of four districts; Attock (355m), Jhelum (233m), Rawalpindi (508m) and Chakwal (498m) is located at latitude 30°-34° N and longitude 70°-74° E in the northern Punjab province. It is a rain-fed zone covering an area of about 13,000 km², representing 2.9% of the country. The Jhelum and Indus River bounded the east and west, while the Kala Chita Range, Salt Range and Murree Hills are on northern and southern side of Pothwar Plateau, having an overall plain to moderately undulating ground, separated by valleys and hills, making the area suitable as pasture land. Climatically, Pothwar Plateau is a sub-tropical continental with erratic rainfall pattern received about 60-70% of the total rainfall (Nizami *et al.*, 2004) and average temperature of 22.5°C. The altitude and agriculture practices in the selected area support the breeding system of warble fly.

Experimental design

A total of 1000 goats belonging to three different breeds (Jattal, Beetal and Tedy) were examined for the seroprevalence during April 2013 to March 2014. Proportional sampling (100 samples per month) was done based on three different categories (houses, farm houses and veterinary hospitals). The random sampling was done from different areas of four selected districts (Rawalpindi, Jhelum, Attock and Chakwal) of Pothwar region.

Detection/Diagnosis

Morphological diagnosis

From April 2013 to March 2014, several flocks (ranged from 10-300 heads per flock), were examined visually as well as hand palpation at back and flank region for the presence of

nodules or lesions. The number of nodules were counted to measure the intensity of infestation.

Serologic diagnosis

For serodiagnosis, the blood samples (1000) were taken from selected animals on monthly basis (regardless of infestation) during the study period. The blood samples were collected from jugular vein of animal using by disposable syringe in non-EDTA coated vacutainer (10 ml). The serum was separated and stored at -20°C for further analysis. Clinically, 100 infested goats with prominent nodules were selected for collection of positive sera, while the negative sera were taken from Lancaster goats, Veterinary Genes and Proteins Laboratory, University of Glasgow, Scotland. The serological test, using commercial ELISA kit was performed according to the prescribed protocols (IDEXX Bovine Hypodermosis Antibody Kit), sensitivity and specificity values were also calculated (Otranto *et al.*, 1999; Faliero *et al.*, 2001; Otranto *et al.*, 2005). The optical density (OD) was recorded at the wavelength of 450 nm.

Epizootiological factors

A questionnaire, based on epizootiological information (age, sex, breed, district, month and topography) was used. The goats were categorized into three age clusters i.e., < 1yr, 1-2 yr and >2 yr old, on the basis of incisor teeth (Khan, 1969). The phenotypic characteristics were considered for breed identification (Hasnain, 1985).

Statistical analysis

The results were analysed by using Pearson's chi-squared (χ^2) test followed by post hoc test (Z-test) with Bonferroni correction in SPSS version 20.0.

RESULTS

The serologic analysis by IDEXX hypodermosis serum ELISA kit and manual palpation procedure were compared for early diagnosis of GWFI from four districts of Pothwar region, Punjab, Pakistan. The sensitivity and specificity of ELISA kit was 90%. The prevalence was 18.5% (185/1000) and 11% (110/1000) by ELISA kit and palpation method, respectively. Higher prevalence of 30.8% and 16.8% was found in Jhelum district while Attock district showed the lowest infestation rate of 6% and 5.2% for commercial ELISA Kit and conventional method, respectively (Table 1 & Fig. 1). Both the diagnostic methods showed significant difference ($p < 0.05$) in the prevalence of GWFI between the four districts.

The prevalence of GWFI, by serological and conventional method in different months revealed a significant difference. Both diagnostic methods showed high prevalence (44%) in December as shown in Table 2 & Fig. 2. The 1st instar larvae were found from July-September, 2nd instar larvae from mid-September to November and 3rd instar larvae were seen from December to February. The nodules start appearing in October to December and last until the end of February. Month-wise analysis by ELISA showed peak in optical density (OD) values in December which gradually decreases with the end of winter season.

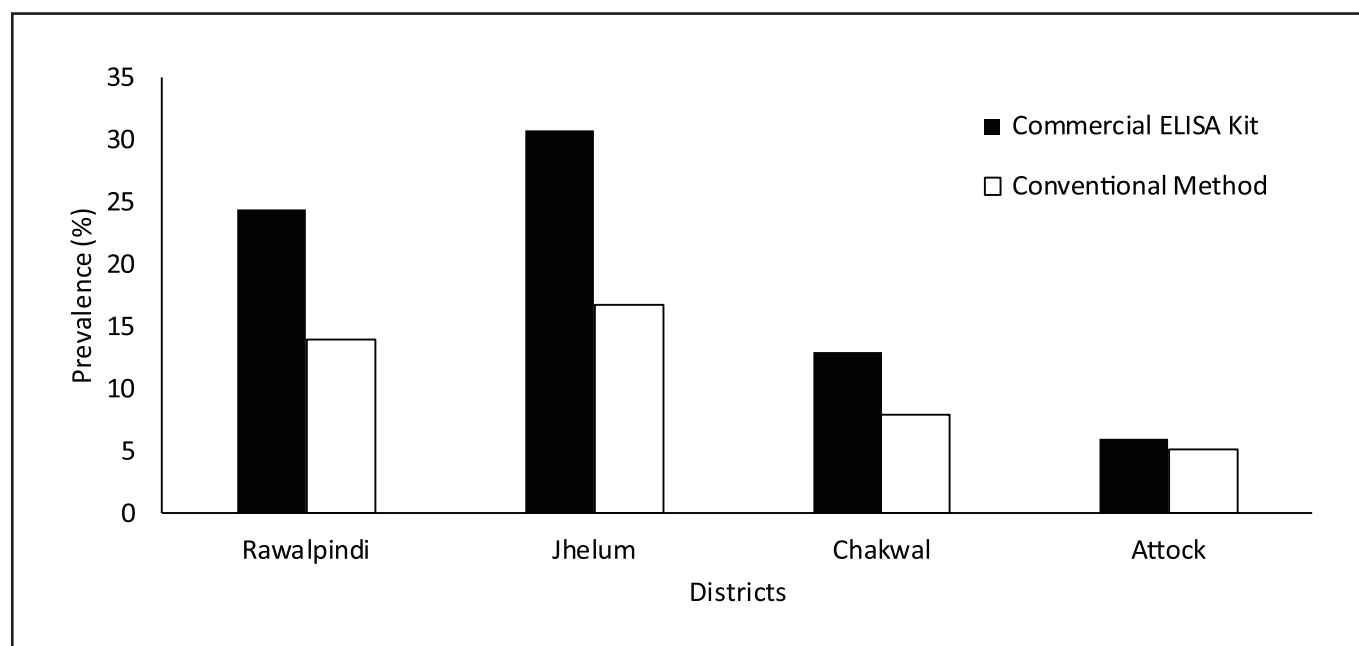
According to sex, the prevalence by conventional method was 11% (63/572) and 10.98% (47/428) whereas, ELISA kit showed 18.8% (108/572) and 17.9% (77/428) seroprevalence in female and male animals, respectively (Table 3 & Fig. 3). High seroprevalence rate was observed in females than males.

Table 1. District wise prevalence of GWFI using commercial ELISA kit and conventional palpation method in Pothwar Region, Punjab, Pakistan

District	Animal Examined	Diagnostic Methods					
		Conventional method			Commercial ELISA kit		
		Non-infested	Infested	Percentage (%)	Non-infested	Infested	Percentage (%)
Rawalpindi	250	215	35	14%	189	61	24.4%
Jhelum	250	208	42	16.8%	173	77	30.8%
Attock	250	237	13	5.2%	235	15	6%
Chakwal	250	230	20	8%	218	32	13%
Total	1000	889	110	11%	815	185	18.5%

Chi-square (χ^2) Test: Hand palpation (F = 20.6, df = 3, p < (0.05).

Chi-square (χ^2) Test: Commercial ELISA kit (F = 62, df = 3, p < (0 .05).

**Figure 1.** District wise prevalence of GWFI by conventional and commercial ELISA kit method in Pothwar region, Pakistan.**Table 2.** Month wise prevalence of GWFI in Pothwar region, Pakistan (2013-14)

Month	Animal examined	Positive	Percentage (%)	Z-test
June (A)	100	0	0	
July (B)	100	0	0	
August (C)	100	0	0	
September (D)	100	0	0	
October (E)	100	7	7	
November (F)	100	21	21	E, I, J
December (G)	100	44	44	E, F, I, J
January (H)	100	29	29	E, I, J
February (I)	100	8	8	
March (J)	100	1	1	
Total	1000	110	11%	

Z-test, revealed the prevalence of GWFI in month of Nov. (F) and Jan. (H), vary significantly from Oct. (E), Feb. (I) and Mar.(J): whereas, Dec. has maximum prevalence (44%).

The prevalence of GWFI in three age groups (<1 yr, 1-2 yr and >2 yr) using traditional method was 16.4% (85/518), 6.5% (15/231) and 4% (10/251), respectively while seroprevalence based on ELISA kit was 28% (144/518), 10.8% (25/231) and 6.4% (16/251) in <1 year, 1-2year and >2 year, respectively (Table 3 & Fig. 4). According to the results, higher infestation was observed in goats of age group (<1 year) as compared to animals (> 2 years) in all studied areas. Breed wise prevalence was investigated on monthly basis. Statistically, no significant relation was found in the prevalence and seroprevalence of GWFI between the breeds (Jattal, Beetal and Tedy). High prevalence of breeds by clinical method was 12% (41/349) whereas the serodiagnosis revealed the prevalence of 21.2% (74/349) in beetal breed (Table 3 & Fig. 5).

Topographically, three different locations viz., plain, hilly and semi-hilly were surveyed. The prevalence by palpation method was 9.5% (24/253), 30.5% (22/72) and 9.5% (64/675) in plain, hilly and semi-hilly areas, respectively. Whereas, the three topographical locations showed the seroprevalence of 14% (35/253), 33.3% (24/72) and 19% (126/675), respectively (Table 3 & Fig. 6).

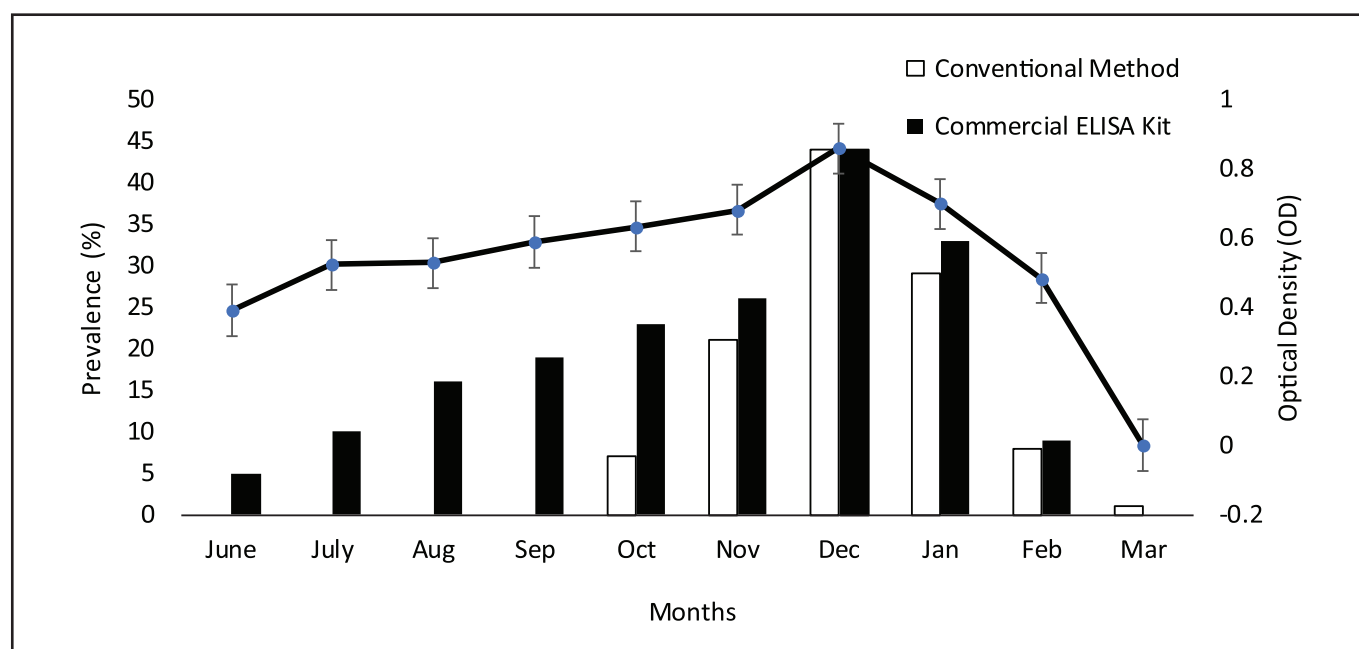


Figure 2. Month wise prevalence of GWFI by conventional and commercial ELISA kit method in Pothwar region, Punjab, Pakistan.

Table 3. Possible risk factors for the seropositivity of GWFI and their p-values based on Chi-square analysis (χ^2)

Factor	Level	Examined animals	Diagnostic Techniques					
			Palpation method			ELISA		
			Non-infested animals	Infested animals	p-value	Non-infested animals	Infested animals	p-value
Sex	Male	428	381 (89.1%)	47 (10.9%)	p=0.98	350 (82.1%)	77 (17.9%)	p=0.89
	Female	572	509 (89%)	63 (11%)		464 (81.2%)	108 (18.8%)	
Age	<1yr	518	433 (83.6%)	85 (16.4%)	p<0.05	374 (72%)	144 (28%)	p<0.05
	1-2yr	231	216 (93.5%)	15 (6.5%)		206 (89.2%)	25 (10.8%)	
	>2yr	251	241 (96%)	10 (4%)		235 (93.6%)	16 (6.4%)	
Breed	Jattal	245	220 (90%)	25(10%)	p=0.83	210 (86%)	35 (14%)	p=0.11
	Beetal	349	308 (88%)	42 (12%)		275 (78.8%)	74 (21.2%)	
	Tedy	406	362 (89%)	44 (11%)		330 (81.3%)	76 (18.7%)	
Topography	Plain	253	229 (90.5%)	24 (9.5%)	p<0.05	218 (86%)	35 (14%)	p<0.05
	Hilly	72	50 (69.5%)	22 (30.5%)		48 (66.7%)	24 (33.3%)	
	Semi-hilly	675	611 (90.5%)	64 (9.5%)		549 (81%)	126 (19%)	
Season	Summer	400	400 (100%)	0	p<0.05	350 (87.5%)	50 (12.5%)	p<0.05
	Winter	600	490 (81.6%)	110 (18.4%)		465 (77.5%)	135 (22.5%)	

DISCUSSION

In current study, higher seroprevalence was reported by using ELISA Kit (IDEXX Hypodermosis serum antibody test) than conventional method in Pothwar region of Pakistan. This difference might be due to antibody mediated host immune-response, as the first instar larvae detected by ELISA, at early stage were later killed by the host defence system and consequently remain undetected on manual examination. Our results correlates with previous findings reported from different areas of Pakistan (Shah, 1981; Arshad *et al.*, 2014) and other parts of the world (Otify & Mansour, 1994; Abo-Shehada *et al.*, 2006; Oryan *et al.*, 2009). However, no previous study by using serological assays was conducted yet on GWFI in Pothwar region, Pakistan.

The results confirmed the efficacy of Hypodermosis ELISA kit for GWFI. Our findings were in accordance with Navidpour *et al.* (2007), reported the sensitivity and specificity of 86.66% and 98.87%, respectively.

The variation in prevalence rate among the districts might be due to management system, grazing patterns, pastures and use of insecticides. Similar findings were reported by Ahmed *et al.* (2012) and Arshad *et al.* (2014). The month wise study supported biological cycle as December has highest the seroprevalence whereas, prior to June no antibodies were observed in the animal body, as hypodermosis was in different development stage. Serologically, the WFI was seen in sera samples from June and July (summer season, 12.5%). Whereas, manually the warble fly infestation was found in November until February (winter,

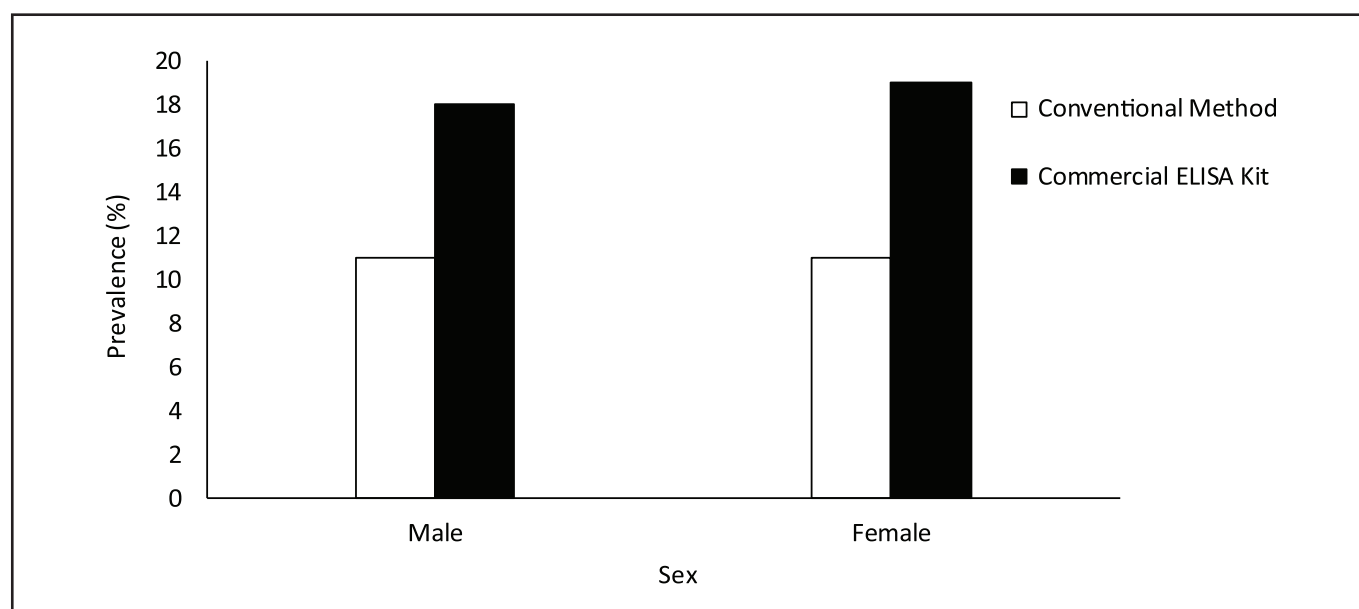


Figure 3. Sex based prevalence of GWFI by conventional and commercial ELISA kit method in Pothwar region, Punjab, Pakistan.

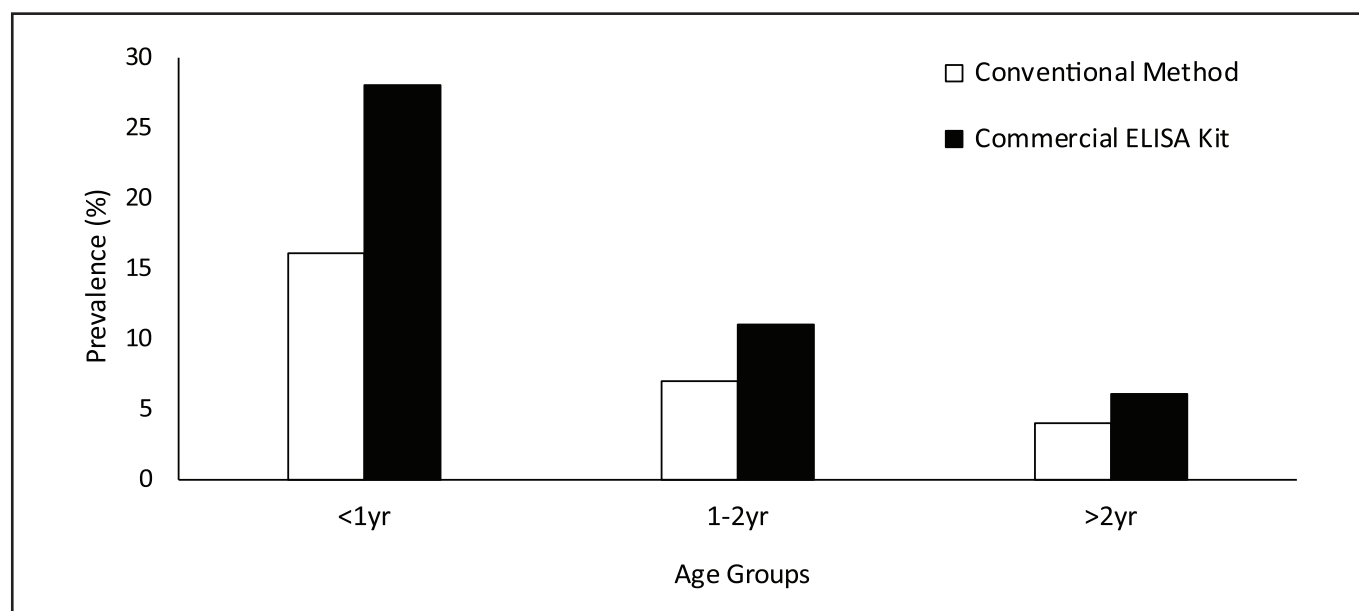


Figure 4. Age wise prevalence of GWFI by conventional and commercial ELISA kit method in Pothwar region, Pakistan.

18.4%) after appearance of nodules on the flank and back of the goats. The highest infestation in the month of December may be attributed to the presence of third stage larvae in infested animals. The skin perforation appeared in January and nodule number began to disappear in February resulting in decreased infestation rate in March, indicating pupation period in the region. These findings were in conformity with other studies (Khan *et al.*, 1994; Khan *et al.*, 2006; Oryan *et al.*, 2009; Tavassoli *et al.*, 2010; Yadav *et al.*, 2011).

The prevalence of warble fly infestation regarding epizootiological factors like age, breed, topography and season was monitored. The clinical and sub-clinical methods revealed no difference among both sexes. Our research findings were in line with previous studies (Rahbari & Ghasemi, 1997; Yadav *et al.*, 2006; Oryan *et al.*, 2009; Domínguez *et al.*, 2010; Oryan & Bahrami, 2012; Arshad *et al.*, 2014; Jan *et al.*, 2014).

The prevalence of GWFI among different host breeds was monitored, showing low infestation in Jattal (Hairy) breed. The reason of lower prevalence in Jattal breed might be that they were kept as small flock by marginal farmers in Punjab or either kept on partial grazing and stall feeding. Whereas, in Punjab Tedy and Beetal goat breeds are preferred over Jattal by farmers and kept on free grazing making them prone to more infestation. Previously, no significant difference among the breeds was detected (Tavassoli *et al.*, 2010; Radfar & Hajmohammadi, 2012). In contrary, higher infestation in Desi (Tedy) breed might be due to their weak immune response (Arshad *et al.*, 2014). Further investigations are required to evaluate the immunity of each breed against GWFI.

Among different age groups, higher seropositivity was observed in young animals of age group (<1 year) as compared to older animals (> 2 years) in all studied areas revealing

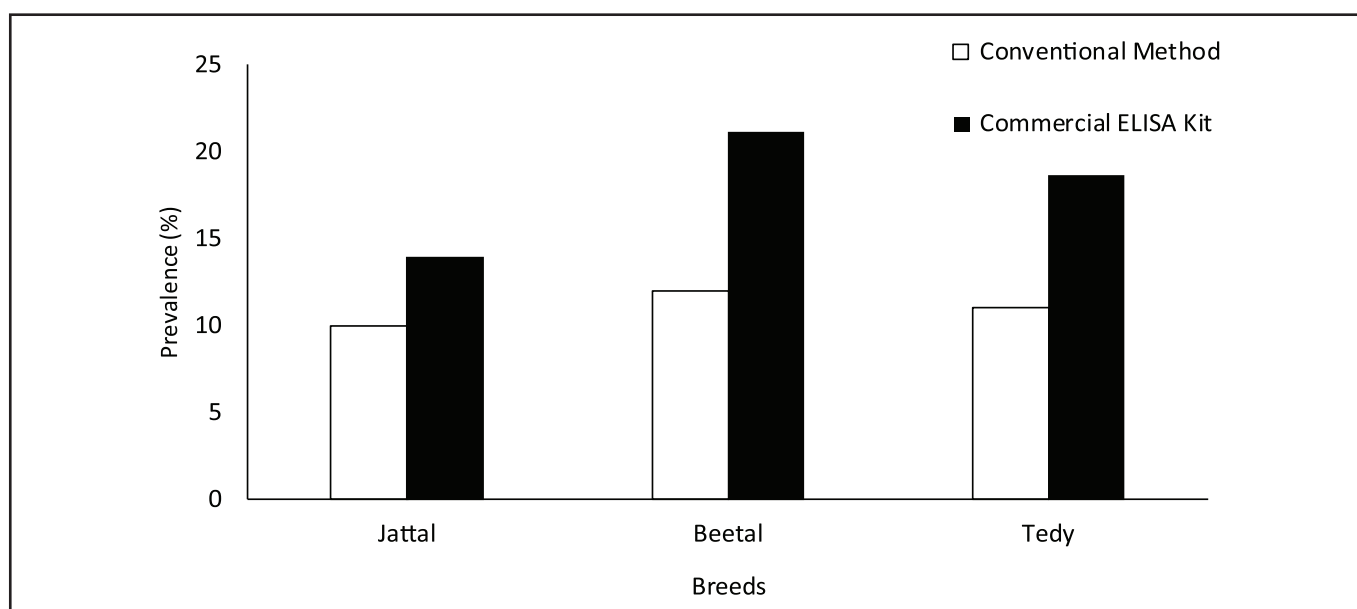


Figure 5. Breed wise prevalence of GWFI by conventional and commercial ELISA kit method in Pothwar region, Pakistan.

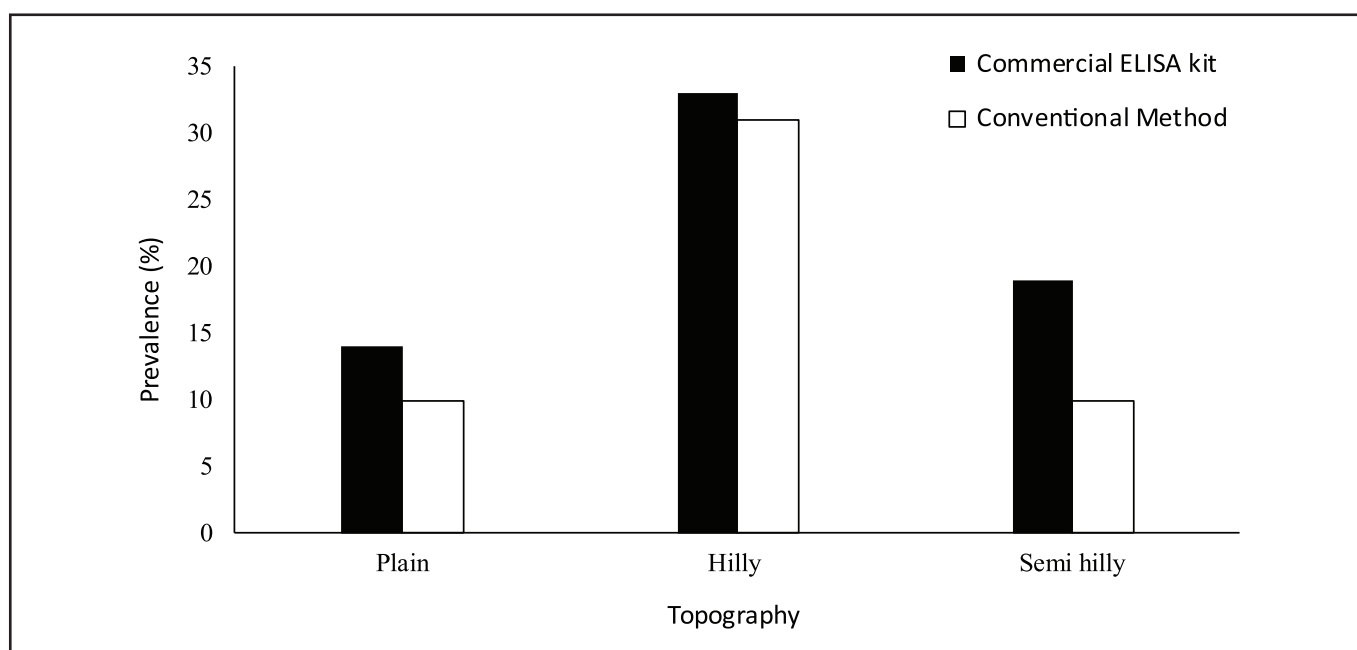


Figure 6. Location wise prevalence of GWFI by conventional and commercial ELISA kit method in Pothwar region, Pakistan.

a significant different ($p < 0.05$). Our findings of lower prevalence in older animals were in accordance with the previous studies (Otranto & Puccini, 2000; Oryan *et al.*, 2009; Hassan *et al.*, 2010; Arshad *et al.*, 2014) whereas, low seropositivity in the animals over 3 years of age could be attributed to the development of acquired resistance in response to previous exposure (Oryan *et al.*, 2009; Hassan *et al.*, 2010; Jan *et al.*, 2014). According to Oryan *et al.* (2009), higher number of infested young goats might be due to their soft skin as compared to the hard and thicker skin of older animals, which serve as a barrier for the penetration of larvae.

Topographically, higher seroprevalence of warble fly infestation was observed in mountainous and semi mountainous lands compared to the plain areas. Higher infestation in hilly areas might be due to favourable climatic

conditions for the larval stages while in mountainous and semi mountainous areas, the agricultural practices were not common as practiced in plain areas. Similar findings were reported by Hasan *et al.* (2007) while in contrary to our findings (Khan *et al.*, 1997; Ayaz, 1998; Jan *et al.*, 2014) reported the variation in prevalence rate.

Commercial ELISA kit, developed for bovine hypodermosis proved a useful diagnostic tool for detection of GWFI at the earlier developmental phase in which animals having antibodies in their blood and without having any nodule or lesion. Whereas, the traditional palpation method diagnosis the infestation at the later stage when too much damage has been done.

It is concluded from the present study that ELISA tool was more specific, effective and accurate diagnostic method than traditional palpation procedure. The age of animal,

season and location were the potential risk factors for GWFI. It is strongly recommended to separate the animals found infected through ELISA test for timely treatment, as not to perpetuate the condition. It is also required to find seroprevalence and risk factors from other areas of the country that helps to reveal the exact status of the infestation in the country.

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Conflict of interest

The authors have no competing interests to declare.

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