

PREVALENCE OF *HAEMONCHUS CONTORTUS* IN NATURALLY INFECTED SMALL RUMINANTS GRAZING IN THE POTOHAR AREA OF PAKISTAN

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ABSTRACT

The present study was carried out to investigate the prevalence and seasonal trend of the *Haemonchus contortus* in sheep and goats in the Potohar areas of northern Punjab, Pakistan from December 2004 to January 2006. Faecal samples collected from 968 sheep and 961 goats of different breeds were examined by the modified McMaster technique using saturated solution of sodium chloride. Results revealed that the infection was significantly ($P<0.05$) higher in sheep compared to goats. The peak infection level was recorded during rainy season (July-October). On the other hand, low infection level was noted from December upto May. In sheep, highest log faecal egg counts (LFECs) were recorded in Islamabad, followed by Attock, Jhelum and Chakwal. However, in goats the LFECs trend was highest in Islamabad, followed by Jhelum, Attock and Chakwal districts. A significant ($P<0.05$) variability in LFECs was noted between sheep and goat breeds from site-site, while no significant difference was observed between breeds at the same site. Hairy (Jattal) goats and Salt-Range (Latti) sheep breeds exhibited significantly reduced LFECs level along with higher packed cell volume (PCV) and haemoglobin (Hb) levels compared to other breeds. Moreover, FAMACHA[®] chart scoring in relation with worm infection (FECs) was more valid in sheep than goats. High prevalence of *H. contortus* in Potohar areas was due to favourable agro-climatic conditions that favour the development and survival of the free-living stages of *H. contortus*. The findings are discussed with regard to their relevance for strategic control of haemonchosis in small ruminants.

Key words: *Haemonchus contortus*, prevalence, agro-climatic conditions, sheep, goats, Potohar area.

INTRODUCTION

The prevalence of gastrointestinal nematodes (GIN) in tropical and subtropical areas has adversely affected the production potential of sheep and goats, leading to countless deaths and insidious economic losses in livestock sector (Al-Quaisy *et al.*, 1987). Among GIN, *Haemonchus contortus* is considered main culprit causing anaemia and hypoproteinaemia in ruminants (Reinecke, 1983). Several epidemiological studies on the GIN infection were carried out to depict the seasonal pattern of haemonchosis in different agro-ecological areas of the world (Gupta *et al.*, 1987; Fritsche *et al.*, 1993; Besier and Dunsmore, 1993; Miller *et al.*, 1998; Hoste *et al.*, 2001; Agyei, 2003; Ng'ang'a *et al.*, 2004).

The seasonal trend in the haemonchosis is influenced by a number of abiotic and biotic factors that dictate the development and survival of pre-parasitic stages of *H. contortus* onto the herbage (Gupta *et al.*, 1988). This situation has highlighted the need to acquire comprehensive epidemiological knowledge of haemonchosis in order to devise appropriate and cost effective strategies to control GIN parasites with timely

anthelmintic treatments in study area. The purpose of this investigation was to study the prevalence of *H. contortus* of sheep and goats at different farms in the Potohar areas of Pakistan. Emphasis was also placed on determining the seasonal pattern of *H. contortus* infection by evaluating the effect of the level of worm infection (FECs), haematocrit values and pallor scoring of ocular mucous membranes by FAMACHA[®] application for identifying animals which require anthelmintic treatment by minimizing indiscriminate and overuse of anthelmintics.

MATERIALS AND METHODS

Study area

The area considered in the present study is commonly known as "Potohar Plateau" comprising Islamabad, Attock, Chakwal and Jhelum districts. The Potohar area is mainly regarded as pasture land and hence its general human populace depends upon maintenance of livestock. The Potohar Plateau is situated between latitude 30 and 34°N and longitude 70 and 74°E. Climatically, this area is further categorized into two sub-zones; first one is sub-humid covering the

Islamabad district, located towards the northern part of Punjab province with an annual rainfall ranging from 500-1000 mm. While the second one is semi-arid (300-500 mm rain fall), located towards the south-western side of Islamabad, comprising the districts of Attock, Chakwal and Jhelum. Weather is divided into four well-marked seasons; Cold season (December to March); Hot season (April to June); Monsoon season (July to September) and Post-Monsoon season (October and November). Native vegetation is characterized by open patches of grasses and forbs species. Climatic data pertaining to maximum, minimum temperature ($^{\circ}\text{C}$) and rainfall (mm) were obtained from the nearby Meteorological stations of experimental sites (Fig. 1).

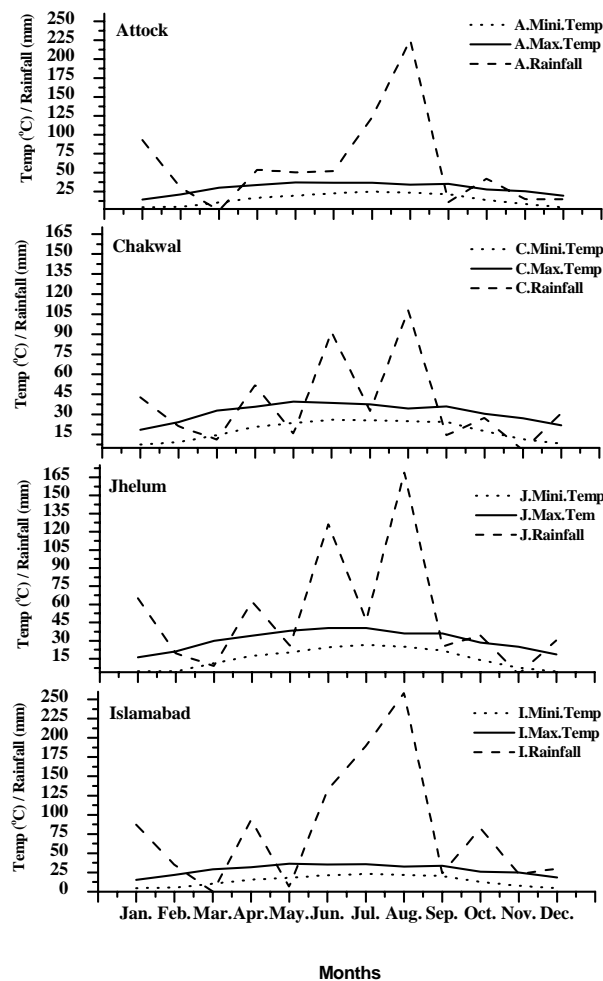


Fig. 1: Mean monthly climatic data at the study sites.

Animals characteristics and management

This one year study (December-2004 to January-2006) was conducted on various public and privately owned small ruminants farms situated along the road

transect. Different animal farms were visited on monthly basis during which 1929 female animals (sheep, 968 and goats, 961) were randomly selected and sampled. These animals belonged to five breeds of sheep viz. Awassi, Afghani (Bulkhi), Pak. Karakul, Afghani x Awassi (cross) and Latti (Salt Range), and another five breeds of goats namely Hairy (Jattal), Beetal (Gujrati), Dwarf (Teddy), Beetal (Faisalabadi) and Beetal x Hairy (cross). Small ruminant farmers in the study area usually follow extensive and semi-extensive farming production systems. The flocks are taken out in the morning for grazing on communal land, roadsides or land along the foot-hills and brought back to their holdings in the evening. Usually flocks live in mud, brick, thatched or mud-plastered houses.

Parasitological protocols

Rectal faecal samples were processed for nematode faecal egg counts (FECs) following the modified McMaster method described by Miller *et al.* (1998), at a sensitivity of 50 eggs per gram (EPG) of faeces. *H. contortus* (L₃) larvae were examined at 10 X magnification and were identified according to the keys and morphological characteristics described by MAFF (1997).

Haematological protocols

Blood samples were taken in EDTA coated vacutainer tubes, from the jugular vein for the determination of PCV and Hb levels, as mentioned by Coffin (1995). Packed cell volume (PCV) was determined by using the micro-haematocrit method. Haemoglobin level was examined by using kit method (AMP diagnostic) on Biochemical system.

FAMACHA[®] chart scoring

FAMACHA[®] eye color chart clearly depicts various categories from healthy to severely anaemic condition. The ocular mucous membranes of the sheep and goats were examined by comparing them with the laminated color chart bearing the picture of sheep conjunctiva (Kaplan *et al.*, 2004). This chart was calibrated into five categories i.e. 1 = red (non-anaemic); 2 = red-pink (non-anaemic); 3 = pink (mildly-anaemic); 4 = pink-white (anaemic) and 5 = white (severely anaemic). All scorings were done on the same day along with faecal and blood samplings.

Statistical analysis

Different variables were analyzed by analysis of variance using SPSS-10. When F-ratio was significant ($P < 0.05$), LSD test was used to compare the means. Data on egg counts were transformed into $[\log_{10}(n+1)]$ to stabilize the variance. No transformation was applied to PCV and haemoglobin data.

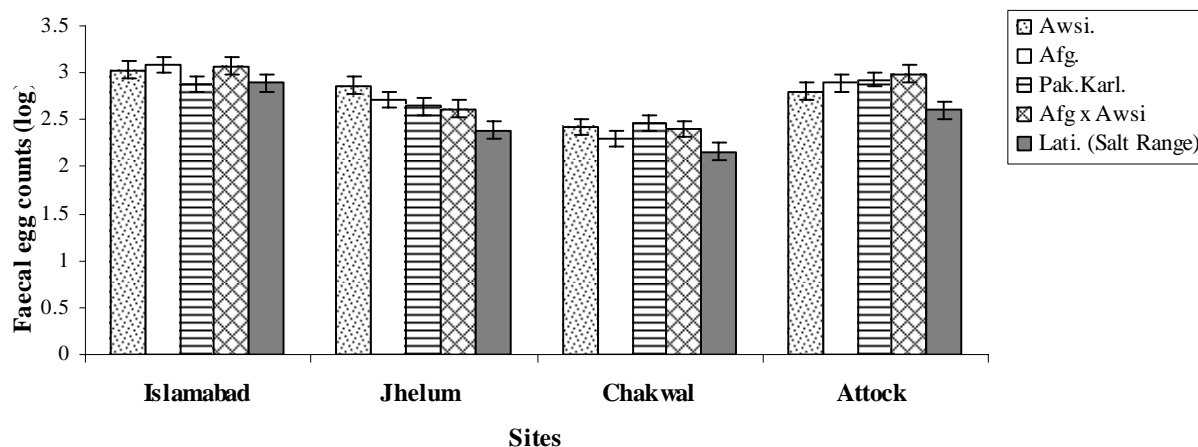


Fig. 2: Intensity of *H. contortus* infection with respect to study area in ewes.

RESULTS

The microscopic study of *H. contortus* larvae showed that they were the medium sized with distinct rounded tail having total length of 630-790 μ . The length from anus to tip of sheath was 119-165 μ .

Intensity of *Haemonchus contortus* infection

Fig. 2 illustrates that in mean log faecal egg count (LFECs), significant difference ($P < 0.05$) was observed for *H. contortus* infection level among different breeds of sheep at different sites. Potency of infection was highest at Islamabad (3.08 ± 0.0) in Afghani sheep, followed by Attock in Afghani x Awassi cross (2.98 ± 0.0) and Jhelum district in Awassi sheep (2.86 ± 0.0). Results showed that there was consistent fluctuation

regarding site-by-breed interaction in maximum mean LFECs. However, minimum mean LFECs was recorded at all studied sites in Islamabad (2.89 ± 0.0), Jhelum (2.39 ± 0.0), Chakwal (2.16 ± 0.0) and Attock (2.58 ± 0.0) in Latti sheep. Moreover, different breeds did not differ significantly at the same site.

Among different goat breeds at same site, no significant difference in mean LFECs was observed, however level of infectivity (FEC) differed significantly ($P < 0.05$) among different breeds at different sites (Fig. 3). Overall infection order with highest mean LFECs was recorded in Beetal x Hairy cross at Islamabad (2.58 ± 0.1), followed by Jhelum and Attock districts, while the lowest level of *H. contortus* infection was recorded in Teddy goats at Chakwal district (1.53 ± 0.0).

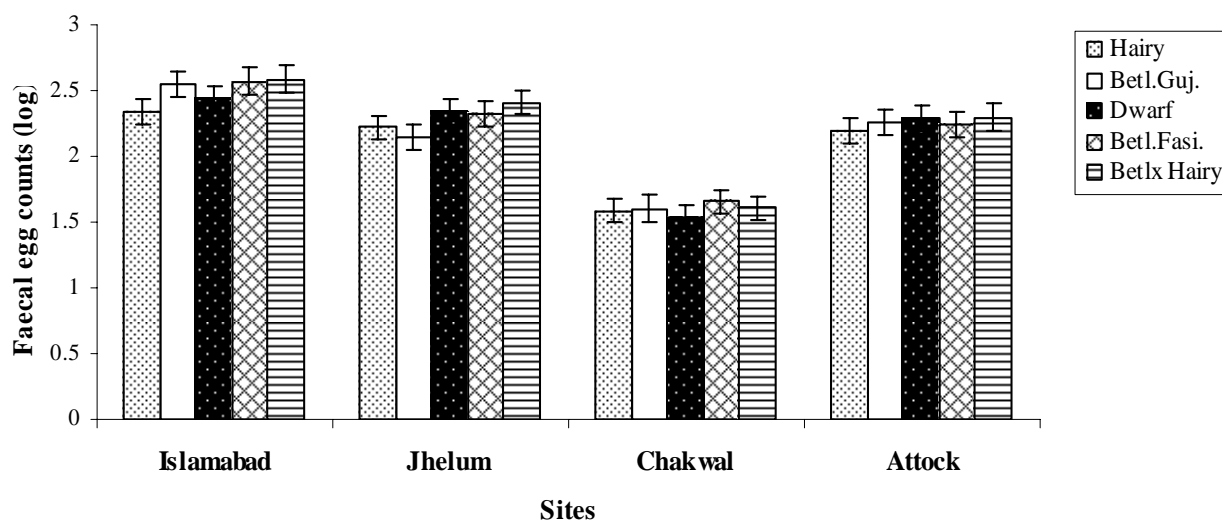


Fig. 3: Intensity of *H. contortus* infection with respect to study area in goats.

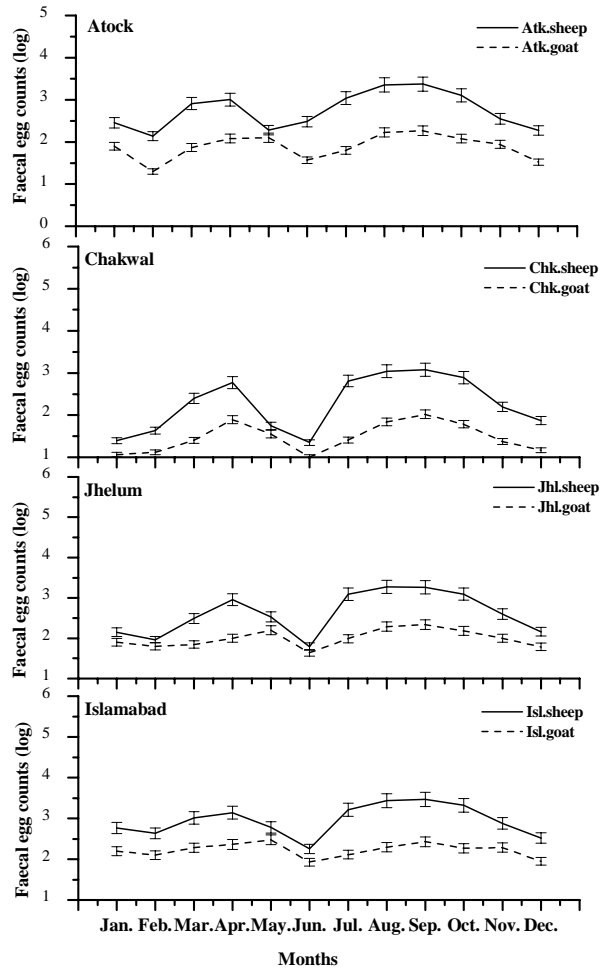


Fig. 4: Relative distribution of *Haemonchus contortus* between sheep and goats throughout the study period.

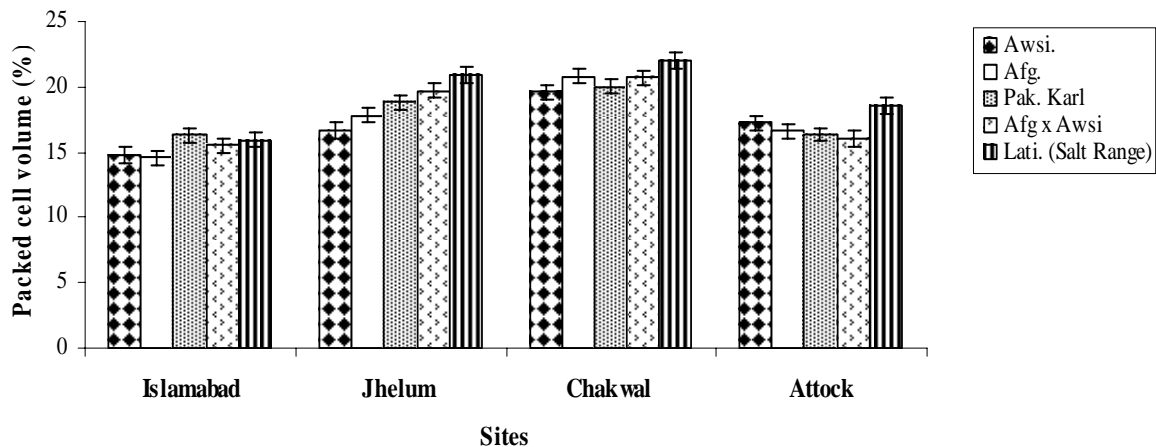


Fig. 5: Distribution pattern of packed cell volume in sheep breeds.

Haemonchus contortus prevalence

Month-wise distribution pattern of *H. contortus* revealed a significant difference among different sites in small ruminants throughout the year (Fig. 4). However, incidence of *H. contortus* infection was higher in sheep ($P < 0.05$) than goats. Both host species showed a clear trend of seasonal *H. contortus* pattern, with peak of infection level from July-October. From October and May onwards, the degree of infection fell off rapidly to reach its lowest level in December and June, respectively. Moreover, among sheep and goats, maximum mean LFECs were recorded in September (3.46 ± 0.1) and August (2.29 ± 0.1) at Islamabad, while minimum level in June (1.35 ± 0.1) and January (1.06 ± 0.1) at Chakwal.

Haematological profiles

A significant difference was found ($P < 0.05$) between packed cell volume (PCV) and haemoglobin (Hb) levels among different sheep breeds at different sites (Fig. 5). The highest PCV was recorded at Chakwal ($22.04 \pm 0.6\%$) in Latti sheep, followed by Jhelum and Attock, while the lowest level was at Islamabad in Afghani sheep ($14.60 \pm 0.5\%$). The highest level of Hb was recorded in Afghani x Awassi (cross) at Chakwal (11.43 ± 0.1 g/dl), followed by Jhelum, and Attock, while the lowest level was observed at Islamabad in Afghani sheep (7.22 ± 0.1 g/dl).

No significant difference ($P > 0.05$) was observed among different goat breeds of the same site but it differed significantly ($P < 0.05$) among different sites (Fig. 6). The highest PCV ($18.82 \pm 0.2\%$) and Hb (9.53 ± 0.21 g/dl) levels were recorded at Chakwal in Beetal x Hairy cross, while the lowest values of PCV ($12.60 \pm 0.30\%$) and Hb (4.90 ± 0.24 g/dl) were recorded at

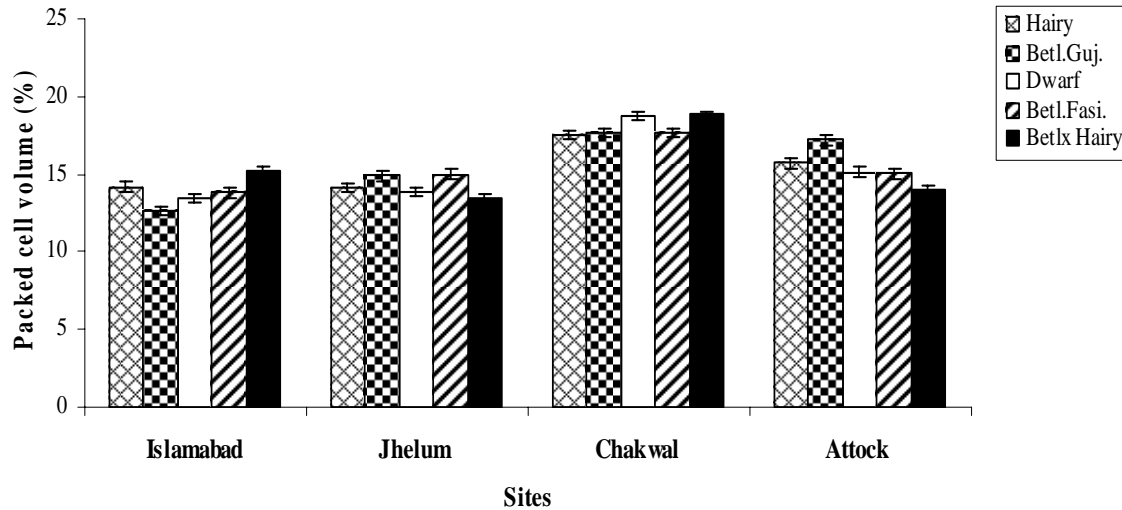


Fig. 6: Distribution pattern of packed cell volume in goat breeds at different sites.

Islamabad in Beetle Gujrati. Moreover, PCV and Hb showed positive significant correlation ($P < 0.05$) in all sheep and goats.

Evaluation of FAMACHA® chart

Results revealed that in sheep with an eggs per gram (EPG) level of 1050 - 2000, most of the animals fell in category 4 with pink-white eye colour (Table 1). Similarly, with EPG level above 2050, animals fell in category 5, having white eye colour showing severe anaemic condition. Animals showed anaemic and severe anaemic conditions with the constant increase in the level of worm infection. The results elucidated a relationship between EPG level and FAMACHA® categories. Moreover, FAMACHA® scoring had significantly positive correlation ($P < 0.05$) with level of *H. contortus* infection (FECs) in case of sheep at different experimental sites.

In goats, with an increase in worm infection level, there was an irregularity in the FAMACHA® scoring (Table 2). Therefore, FAMACHA® chart categories had no significant correlation ($P > 0.05$) with amount of FECs in case of goats throughout the study period.

DISCUSSION

The results revealed that the infected animals (sheep and goats) harbour *H. contortus* infection throughout the year at all the studied sites with varied incidence. It could be inferred from the infection level that the permanent flocks in Potohar areas had substantial worm burdens of *H. contortus*. Gupta *et al.* (1988) also reported that larger infection might be due to conducive environmental temperatures (19°C and 37°C) prevalent in the area. On the basis of FECs, in sheep the infection was significantly higher (FECs 50-4550) compared to goats with FECs range of (50-3400). This finding is in conformity with previous workers (Jacquet *et al.*, 1992; Fritsche *et al.*, 1993; Ndao *et al.*, 1995), who have also reported that sheep generally harbour more GIT nematodes than goats. The plausible explanation might be the fact that sheep are generally grazer in their feeding habit and usually graze very close to the soil which might be helpful in the acquisition of more infective larvae (L_3) of *H. contortus* from the contaminated herbage. On the other hand, goats browse on shrubs and small trees where translation of infective larvae to such a height seems to be impossible.

Table 1: Various sets of FECs ranges in sheep for evaluation of FAMACHA® system

FAMACHA® categories	Faecal egg counts (FECs) ranges								
	50-500	550-1000	1050-1500	1550-2000	2050-3000	3050-3500	3550-4000	4550-4500	4500-5000
1.	109	0	0	0	0	0	0	0	0
2.	92	91	0	0	0	0	0	0	0
3.	29	154	40	30	0	0	0	0	0
4.	0	0	98	101	7	0	0	0	0
5.	0	0	2	19	49	51	30	6	3

Table 2: Various sets of FECs ranges in goats for evaluation of FAMACHA® system

FAMACHA® categories	Faecal egg counts (FECs) ranges						
	50-500	550-1000	1050-1500	1500-2000	2050-2500	2550-3000	3050-3500
1.	46	0	0	0	0	0	0
2.	119	8	7	2	0	0	0
3.	205	59	40	27	24	12	0
4.	14	93	31	54	36	28	19
5.	0	1	11	20	19	20	2

As apparent from the results, there were substantial differences between-site in overall intensity of *H. contortus* among different breeds. Highest incidence of infection level (FECs) in sheep was found at Islamabad compared to Attock, Jhelum and Chakwal. While goats at Islamabad site got more infection as compared to Jhelum, Attock and Chakwal. These differences in infection level might be due to varied acquisition of worms acquired by these hosts from these areas. These results are in agreement with those of McKenna (1988) and Fakae (1990), who demonstrated a direct association between the incidence and the level of infection. The incidence of *H. contortus* infection recorded at these sites might be determined principally by the availability of infective larvae on herbage. This in turn depends on the development and spread of eggs in faeces into infective larvae (L₃) stage on pasture, a process completed within required temperature and humidity. Furthermore, higher incidence in Islamabad area may be coupled with adequate rainfall and relatively high humidity that this site might receive compared to other sites. The low incidence in Chakwal district recorded in this survey seems to be due to low mean rainfall that this area received during the entire year.

Results revealed a significant difference between different breeds both in sheep and goats for resistance to GIN *H. contortus*. These findings are consistent with those of Courtney *et al.* (1985) and Baker *et al.* (1999), who observed genetic differences among breeds and within-breed, resulting in variable resistance to infection by GIT nematodes. It is further revealed that differences among breeds in LFECs values and their effect on PCV and Hb levels could have been due to difference in mode of acquisition of infective larvae of *H. contortus* from contaminated herbage and their subsequent development into adult worms in various host breeds. Furthermore, it is generally accepted that immune response plays a vital role in the demonstration of inheriting resistance (Costa *et al.*, 2000; Vanimisetti *et al.*, 2004; Good *et al.*, 2006). The resistant hosts have the capability to mount a more effective response to parasite than a susceptible host does (Barger, 1989). In the present study, Latti (Salt Range) sheep breed and

Hairy (Jattal) goats, both breeds native of the Potohar areas, had comparatively higher PCV and Hb levels with low LFECs levels. These two breeds seemed to have become well-adopted to the GINs in Potohar areas. However, genetic and immunological display of these breeds with relation to GIT nematodes needs to be explored further, ultimately the benefit of exploiting the genetic variations in resistance to GIT nematodes is an excellent approach towards accomplishing sustainable GIT nematode in future control management.

Variable PCV and Hb levels recorded in various animal breeds during this study might have occurred as a result of physiological effect due to undernourishment and non-availability of supplementary feed to these animals. For low PCV and Hb levels, Torres-Acosta *et al.* (2006) have suggested that non-supplemented animals are capable of a high degree of compensation to protect their internal homeostasis and to preserve normal blood levels especially under condition of heat stress. This potential is to be carried out in semi-arid condition of Pakistan where long dry condition persists for two-three month in a year. However, the present study shows that animals harbour significant number of *H. contortus* worms as depicted by increased LFECs in these animals. This is in agreement with Wallace *et al.* (1996). The epidemiological studies in Potohar areas depicted that higher numbers of *H. contortus* egg were shed in faeces from July to October in adult sheep and goats. Similar findings were made by Misra *et al.* (1974) and Gupta *et al.* (1987). The high LFECs are probably due to heavy contamination by animals, which gives rise to a higher worm-burden in animals.

The FAMACHA® system may be used to correctly mark those animals which require anthelmintic treatment and it is concluded that this system can prove to be a valuable tool for decision-making to control *H. contortus* in small ruminants, mostly in sheep. This study revealed highly significant correlation between FEC and FAMACHA® eye scoring. Further testing of the FAMACHA® clinical assay should also be pursued in small ruminants as its application limits the spread of anthelmintic resistance and can also help to improve the genetic resistance.

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