Association between epidemiology and haematophagous behaviour of *Haemonchus contortus* and *Ostertagia ostertagi* infecting sheep of Kashmir Valley, India

Irfan-ur-Rauf Tak, Jehangir Shafi Dar, B. A. Ganai* and M. Z. Chishti

Centre of Research for Development, University of Kashmir, Srinagar 190 006, India

Haemonchus contortus and Ostertagia ostertagi are predominantly sheep parasites and majority of the pathological effects they cause are due to their bloodfeeding behaviour. This study was carried out to ascertain the prevalence of these two parasites in sheep of Kashmir Valley, India and to determine effect on haematological parameters of sheep. *H. contortus* was more prevalent than *O. ostertagi*; the infection was found to be higher in summer, lower age groups and males. As far as the effect on haematology is concerned, haemoglobin concentration, packed cell volume and red blood cell count showed significant decrease and white blood cell count showed significant increase in case of infected sheep.

Keywords: Epidemiology, *Haemonchus contortus*, haematophagous behaviour, *Ostertagia ostertagi*, sheep.

PARASITISM is considered as the most challenging problem to livestock industries with an approximate loss of £1000 million per annum¹. Parasites acquire their food from their hosts, causing poor health and stress in the host organism that leads to an increased vulnerability to other diseases. The widespread emergence of anthelminticresistant strains of the highly pathogenic parasitic nematode Haemonchus contortus and other economically important parasites has resulted in the need to develop unconventional control strategies². H. contortus and Ostertagia ostertagi are mostly pathogenic to sheep and cause effects ranging from mild anaemia to mortality in young animals because of their blood-feeding behaviour. Very little work has been carried out on their possible control in the Kashmir Valley, India. During recent times, studies has been carried out on the anthelmintic resistance shown by different kinds of parasites besides the antiparasitic efficacy of herbs and other important plants^{3,4}. This has led to attempts to better understand the biology of these parasites because of the growing concern of residual chemicals in the environment and food chain. Several inter-related disciplines are needed for the efficient control of these parasites. There is an immediate need for basic parasitological techniques to be employed

1776

and this ultimately will enable the maintenance of parasite cultures.

The anti-parasitic market has been among the fastgrowing sectors of the overall US\$18 billion animal health market during the last decade. There is a rising interest in the development of safe and successful control strategies because the drugs for the treatment of parasites of livestock continue to dominate this sector because of consumer demands for chemical-free food⁵. Besides the parasitic infections which have been shown to cause direct economic losses due to reduced animal production, one more dimension is added by the fact that numerous parasitic infections can be transmitted to humans by the phenomenon of zoonoses. An effective control of parasitic diseases is dependent mainly on available information on local conditions and the strength of additional services that transform this knowledge to the farmer. A thorough study on the epidemiology and prophylaxis of gastrointestinal parasites of ruminants is therefore the need of the hour in order to develop novel techniques for investigation, diagnosis, therapy, management and assessment of risk factors that would help the poor farmers. In view of the above facts, this study was carried out to ascertain the prevalence of these two economically important parasites and also determine their effect on haematological parameters of sheep. This will help us in understanding the basic biology of the parasites and ultimately prove to be a useful tool in devising an effective control strategy against these parasites.

The present work was carried out to study the prevalence of infection and also to study the effect of *H. contortus* and *O. ostertagi* on haematological parameters of sheep. Guts that were naturally infected were obtained from slaughtered sheep on the day of slaughter and were carefully examined, particularly the abomasums part and the parasites were collected. Next the parasites were separated into *H. contortus* and *O. ostertagi* based on the standard body lengths: *H. contortus*: female (18–30 mm), male (10–20 mm) and *O. ostertagi*: (~10 mm), and general morphology⁶.

It was made sure that during the entire period of collection, the season of collection, age as well as gender of the host were carefully noted down. The prevalence of *H. contortus* and *O. ostertagi* was then estimated after the collection was completed. The overall, seasonal, age-wise and gender-wise prevalence was calculated using the formulae

Overall prevalence

$$= \frac{\text{Number of specimens that are infected}}{\text{Observed number of specimens}} \times 100$$

Seasonal prevalence

 $= \frac{\text{Infected number of hosts in a particular season}}{\text{Infected number of hosts in that season}} \times 100.$

CURRENT SCIENCE, VOL. 113, NO. 9, 10 NOVEMBER 2017

^{*}For correspondence. (e-mail: bbcganai@gmail.com)

Age-wise prevalence

 $=\frac{\text{Infected number of hosts of a particular age group}}{\text{Number of observed hosts of that age group}} \times 100.$

Gender-wise prevalence

 $=\frac{\text{Infected number of hosts of a particular gender}}{\text{Number of observed hosts of that gender}} \times 100.$

Blood was collected from the same animals whose faecal samples were found to be positive for parasitic infection and all the important information was recorded on a regular basis. Blood was drawn under aseptic condition by vein puncture with the help of a disposable syringe and gradually transferred to a screw-capped sterile test tube in order to avoid haemolysis⁷. All the blood samples were then labelled with proper identification number and date of collection. The samples were left for about 1 h for blood clotting to occur. The clotted blood was then removed with a fine loop and the samples were centrifuged at 3500 rpm for at least 5 min. The serum was finally aspirated with a pasture pipette and transferred to a screw-capped vial and finally stored at -20° C till further analysis.

For other haematological parameters, i.e. estimation of haemoglobin (Hb), red blood cell (RBC) count, white blood cell (WBC) count and packed cell volume (PCV), blood samples were collected from the animals in vials containing EDTA as anticoagulant using the methods as described in the literature^{8,9}. Haemoglobin was estimated by cyanomethaemoglobin method. RBC count estimates the number of red blood cells in a cubic millimetre of blood. An improved Neubaurs chamber was used for counting RBC. WBC count (total leukocyte count, TLC) estimates the total number of WBC per cubic millimetre of blood; this was also done in the same manner as RBC count. For leukocyte classification, the nomenclature of England and Bain¹⁰ was followed. For differential leukocyte count (DLC), a thin blood film was prepared by spreading a blood drop equally on a clean, grease-free slide using a smooth-edged spreader. Modification of Romanowsky's stain namely Leishman's stain was used¹¹. For Giemsa staining, the air-dried blood smears were prefixed with acetone-free methanol for 5 min (ref. 12). The DLC results obtained were compared with the estimated normal values reported in other studies^{13,14}

During the study period, a total of 789 sheep abomasums were examined, out of which 569 (72.11%) were found to be infected. Of these, it was seen that 553 were infected with *H. contortus* and 496 were infected with *O. ostertagi*, while 447 (56.6%) had mixed infection. It was also seen that the number of individual parasites found varied from animal to animal. Therefore, overall prevalence of *H. contortus* in sheep was 70.08% and that of *O. ostertagi* was 62.86% (Figure 1).

CURRENT SCIENCE, VOL. 113, NO. 9, 10 NOVEMBER 2017

The seasonality of infection in the present study revealed the highest prevalence in summer and lowest in winter. Prevalence in spring and autumn fell between summer and winter. During winter months 191 sheep were examined, out of which 93 were infected, giving a prevalence of 48.6%. A total of 206 sheep were examined during spring season, of which 161 were infected, showing a prevalence of 78.15%. Also, a total of 221 sheep were examined during summer season, among which 198 were infected, showing prevalence of 89.59%. Similarly, in autumn season, out of 171 sheep that were examined, 117 were infected, giving a prevalence of 68.42%. Thus it was seen that the prevalence was highest in summer followed by spring, autumn and lowest in winter (Figure 2 and Table 1). P = 0.002 was obtained using chi square test, which indicates that the data are statistically significant (P < 0.05).

For parasite screening, 789 abomasae were collected from animals of different age groups, i.e. <1, 1–2, 2–3 and >4 years. Out of the total 212 lambs having age less than 1 year that were examined, 168 were infected with

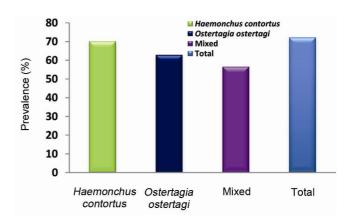


Figure 1. Overall prevalence of H. contortus and O. ostertagi.

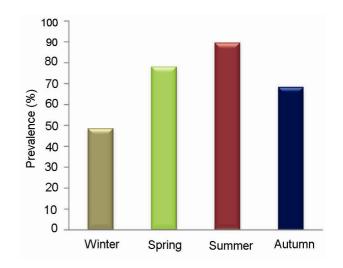


Figure 2. Prevalence of *H. contortus* and *O. ostertagi* during different seasons.

RESEARCH COMMUNICATIONS

H. contortus and 160 with O. ostertagi, showing prevalence of 79.24% and 75.47% respectively. Next 198 were examined in the age group of 1-2 years, of which 146 were infected with H. contortus and 138 with O. ostertagi, showing prevalence of 73.73% and 69.69% respectively. Also, 207 abomasae were examined in the age group of 2-3 years, out of which 138 were infected with H. contortus and 118 with O. ostertagi, showing a prevalence of 66.66% and 57.004% respectively. Finally, 172 abomasae were examined in the age group of >4 years, of which 101 were infected, with H. contortus and 80 with O. ostertagi, showing a prevalence of 58.72% and 46.51% respectively. Thus, it was seen that prevalence was highest in age group of <1 year followed by 1-2 and 2-3 years, and least prevalence was seen in the age group of >4 years (Figure 3 and Table 2). Using chi square test,

 Table 1. Prevalence of H. contortus and O. ostertagi during sheep in different seasons

Season	Number examined	Positive	Prevalence (%)
Winter	191	93	48.6
Spring	206	161	78.15
Summer	221	198	89.59
Autumn	171	117	68.42
Total	789	569	72.11

 Table 2. Prevalence of H. contortus and O. ostertagi in sheep of different age groups

Age group	Number examined	Infected		
		H. contortus (%)	O. ostertagi (%)	
<1	212	168 (79.24)	160 (75.47)	
1-2	198	146 (73.73)	138 (69.69)	
2-3	207	138 (66.66)	118 (57.004)	
>4	172	101 (58.72)	80 (46.51)	
Total	789	553 (70.08)	496 (62.86)	

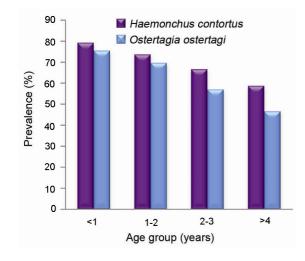


Figure 3. Prevalence of *H. contortus* and *O. ostertagi* in sheep of different age groups.

From both genders, a total of 789 organs were examined. In case of male specimens, 402 organs were taken of which 298 were infected with *H. contortus*, showing a prevalence of 74.12% and 258 were infected with *O. ostertagi*, showing a prevalence of 64.17%. Also, 387 organs were taken from females, of which 255 were infected with *H. contortus*, showing prevalence of 65.89% and 238 were infected with *O. ostertagi*, showing prevalence of 61.49%. Thus infection was higher in males compared to females and P = 0.5 shows that the data are statistically significant (Figure 4 and Table 3).

The mean haemoglobin in uninfected sheep during different seasons, viz. winter, spring, summer and autumn was 13.83 ± 0.81 , 14.16 ± 0.68 , 12.85 ± 0.93 and 13.58 ± 0.49 respectively (P = 0.04; Table 4 and Figure 5). In case of infected sheep, it was found to be 10.91 ± 0.58 , 8.66 ± 0.60 , 7.5 ± 0.63 and 9.08 ± 0.60 during winter, spring, summer and autumn respectively (P = 0.001; Table 4 and Figure 5). The results showed slight decrease in haemoglobin concentration in uninfected sheep in summer compared to other seasons. In the case of infected sheep, there was significant decrease in haemoglobin concentration and the effect was prominent in summer followed by spring and the least in winter season.

The mean value for PCV in the uninfected sheep was 41.19 ± 1.54 , 43.53 ± 1.13 , 34.47 ± 1.27 and 38.13 ± 1.44 during winter, spring, summer and autumn respectively (*P* = 0.001; Table 4 and Figure 6). In case of infected

 Table 3. Prevalence of H. contortus and O. ostertagi in male and female sheep

Gender	Number examined	Infected		
		H. contortus (%)	O. ostertagi (%)	
Male	402	298 (74.12)	258 (64.17)	
Female	387	255 (65.89)	238 (61.49)	
Total	789	553 (70.08)	128 (62.86)	

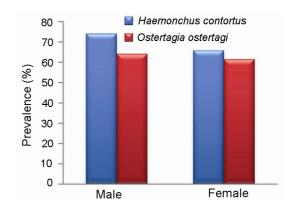


Figure 4. Prevalence of *H. contortus* and *O. ostertagi* in male and female sheep.

	RESEARCH COMMUNICATIONS
Table 4.	Haematological parameters of uninfected and infected sheep

Parameters	$(Mean \pm SD)$				
	Winter	Spring	Summer	Autumn	P value
Uninfected sheep					
Hb (g/dl)	13.83 ± 0.81	14.16 ± 0.68	12.85 ± 0.93	13.58 ± 0.49	0.04
RBC $(10^{6}/\mu l)$	10.76 ± 1.23	15.43 ± 0.97	9.86 ± 1.15	11.36 ± 1.02	0.001
WBC $(10^3/\mu l)$	8.54 ± 1.13	9.42 ± 1.23	7.58 ± 1.06	8.97 ± 1.01	0.055
PCV (%)	41.19 ± 1.54	43.53 ± 1.13	34.47 ± 1.27	38.13 ± 1.44	0.001
Infected sheep					
Hb (g/dl)	10.91 ± 0.58	8.66 ± 0.60	7.5 ± 0.63	9.08 ± 0.60	0.001
RBC $(10^6/\mu l)$	10.62 ± 1.01	8.31 ± 1.23	7.43 ± 1.78	10.42 ± 1.53	0.002
WBC $(10^{3}/\mu l)$	9.12 ± 1.24	10.92 ± 1.06	11.83 ± 1.16	9.85 ± 1.42	0.006
PCV (%)	37.67 ± 2.33	29.21 ± 2.08	25.47 ± 1.89	31.75 ± 2.76	0.001

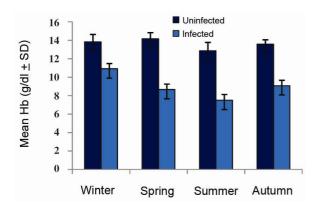


Figure 5. Seasonal variation in haemoglobin (Hb) of infected and uninfected sheep.

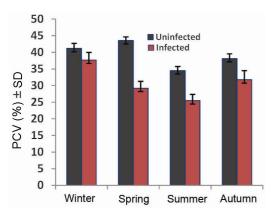


Figure 6. Seasonal variation in packed cell volume of infected and uninfected sheep.

sheep, the mean value during winter, spring, summer and autumn was 37.67 ± 2.33 , 29.21 ± 2.08 , 25.47 ± 1.89 and 31.75 ± 2.76 respectively (P = 0.001, Table 4 and Figure 6). The results showed a decrease in PCV percentage in uninfected sheep during summer compared to other seasons. There was significant decrease in PCV percentage in infected sheep and it was seen that the effect was most prominent in summer and spring seasons compared to other seasons.

18 16 ■ Uninfected Mean RBC's (10⁶/mm³) <u>+</u> SD) 14 Infected 12 10 8 6 4 2 0 Winter Spring Summer Autumn

Figure 7. Seasonal variation in red blood cell count in infected and uninfected sheep.

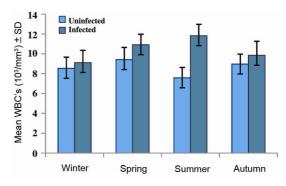


Figure 8. Seasonal variation in white blood cell count in infected and uninfected sheep.

The mean RBC count was 10.76 ± 1.23 , 15.43 ± 0.97 , 9.86 ± 1.15 and 11.36 ± 1.02 in uninfected sheep during winter, spring, summer and autumn respectively. In case of the infected sheep, the mean RBC count was 10.62 ± 1.01 , 8.31 ± 1.23 , 7.43 ± 1.78 and 10.42 ± 1.53 in winter, spring, summer and autumn respectively. The results showed a decrease in RBC count in uninfected sheep during summer compared to other seasons (P =0.001; Table 4 and Figure 7). In the case of infected sheep, the results showed significant decrease in RBC

CURRENT SCIENCE, VOL. 113, NO. 9, 10 NOVEMBER 2017

RESEARCH COMMUNICATIONS

	(Mean ± SD)				
Cells	Winter	Spring	Summer	Autumn	P value
Uninfected sheep					
Neutrophil	34.43 ± 1.08	35.78 ± 1.12	36.17 ± 0.83	35.12 ± 0.98	0.036
Lymphocyte	58.43 ± 0.96	59.85 ± 1.04	60.43 ± 1.24	59.02 ± 1.16	0.025
Eosinophil	7.12 ± 0.91	7.76 ± 0.85	7.97 ± 1.01	7.45 ± 0.76	0.393
Monocyte	3.81 ± 0.81	3.15 ± 0.54	3.01 ± 0.42	3.62 ± 0.41	0.075
Basophil	2.01 ± 0.75	2.34 ± 0.63	2.65 ± 0.88	2.12 ± 0.48	0.425
Infected sheep					
Neutrophil	35.52 ± 1.16	40.54 ± 1.23	44.43 ± 0.84	36.25 ± 1.03	0.001
Lymphocyte	59.86 ± 1.31	68.53 ± 1.18	73.42 ± 1.12	65.2 ± 1.08	0.001
Eosinophil	8.98 ± 1.08	10.42 ± 1.12	11.05 ± 0.86	9.32 ± 0.98	0.007
Monocyte	2.81 ± 0.66	1.89 ± 0.31	1.43 ± 0.89	2.17 ± 0.61	0.012
Basophil	2.05 ± 0.54	2.82 ± 0.71	3.11 ± 0.81	2.32 ± 0.42	0.039

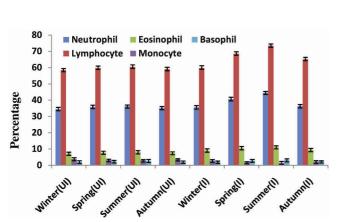


Figure 9. Seasonal variation in differential leukocyte count infected (I) and in uninfected (UI) sheep.

count and the effect was found to be most prominent in summer and spring and less in winter (P = 0.002; Table 4 and Figure 7).

The mean WBC count in uninfected sheep during winter, spring, summer and autumn was 8.54 ± 1.13 , 9.42 ± 1.23 , 7.58 ± 1.06 and 8.97 ± 1.01 respectively (P = 0.055, Table 4 and Figure 8). In case of infected sheep, the mean WBC count was 9.12 ± 1.24 , 10.92 ± 1.06 , 11.83 ± 1.16 and 9.85 ± 1.42 during winter, spring, summer and autumn respectively (P = 0.006; Table 4 and Figure 8). The results showed no such marked variation but a slight decrease in uninfected sheep during summer season. In case of infected sheep there was a significant increase and the effect was most prominent in summer compared to winter season.

The mean percentage value for neutrophils in uninfected sheep during winter, spring, summer and autumn was 34.43 ± 1.08 , 35.78 ± 1.12 , 36.17 ± 0.83 and $35.12 \pm$ 0.98 respectively (P = 0.036, Table 5 and Figure 9). In the case of infected sheep, the mean percentage value for neutrophils during winter, spring, summer and autumn was 35.52 ± 1.16 , 40.54 ± 1.23 , 44.43 ± 0.84 and $36.25 \pm$

1.03 respectively (P = 0.001; Table 5 and Figure 9). The mean percentage value for lymphocytes in uninfected sheep during winter, spring, summer and autumn was 58.43 ± 0.96 , 59.85 ± 1.04 , 60.43 ± 1.24 and 59.02 ± 1.16 respectively (P = 0.025, Table 5 and Figure 9). In the case of infected sheep, the mean percentage value for lymphocytes was 59.86 ± 1.31 , 68.53 ± 1.18 , $73.42 \pm$ 1.12 and 65.2 ± 1.08 in winter, spring, summer and autumn respectively (P = 0.001, Table 5 and Figure 9). The mean percentage value for eosinophils in uninfected sheep during winter, spring, summer and autumn was 7.12 ± 0.91 , 7.76 ± 0.85 , 7.97 ± 1.01 and 7.45 ± 0.76 respectively (P = 0.393, Table 5 and Figure 9). In the case of infected sheep, it was found to be 8.98 ± 1.08 , 10.42 ± 1.12 , 11.05 ± 0.86 and 9.32 ± 0.98 in winter, spring, summer and autumn respectively (P = 0.007,Table 5 and Figure 9). The mean percentage value for monocytes in uninfected sheep during winter, spring, summer and autumn was 3.81 ± 0.81 , 3.15 ± 0.54 , $3.01 \pm$ 0.42 and 3.62 \pm 0.41 respectively (P = 0.075, Table 5 and Figure 9). In the case of infected sheep, it was $2.81 \pm$ 0.66, 1.89 ± 0.31 , 1.43 ± 0.89 and 2.17 ± 0.61 in winter, spring, summer and autumn respectively (P = 0.012), Table 5 and Figure 9). Finally, the mean percentage value for basophils in uninfected sheep was 2.01 ± 0.75 , $2.34 \pm$ $0.63, 2.65 \pm 0.88$ and 2.12 ± 0.48 in winter, spring, summer and autumn respectively (P = 0.425; Table 5 and Figure 9). In the case of infected sheep, it was 2.05 ± 0.54 , 2.82 ± 0.71 , 3.11 ± 0.81 and 2.32 ± 0.42 in winter, spring, summer and autumn respectively (P = 0.039, Table 5 and Figure 9). The results of the present study showed not much variation in DLC in the uninfected sheep, but showed a significant increase in neutrophils and lymphocytes in infected sheep. Slight increase in eosinophils and basophils and slight decrease in monocytes in infected sheep were also observed in the present study.

Similar results have been reported by several other researchers^{15–19}. However, there are also studies reporting

results in contrast to the present study^{20,21}. It may be noted that the differences in the prevalence percentages may be due to the environmental conditions present at the place of collection, climate of the valley, different hosts and may also be attributed to the low frequency of intermediate hosts. In case of seasonal prevalence, it was seen that the infection was more in summer compared to other seasons. Previous studies have also reported highest prevalence in summer compared to other seasons²²⁻²⁴. The higher infection during summer may be attributed to the seasonal/climatic pattern and conditions, as it provides optimum conditions for herbage growth and the required moisture for proper development of the parasites. Also, the hot and humid weather provides optimum conditions for the development and survival of exogenous stages of *H. contortus*²⁵. In winter the lower prevalence percentages may be because the temperature is low and also the atmosphere is dry which might have repressed the development of eggs and larvae. While observing the prevalence in different age groups and in different sexes, it was seen that lower age groups and males were more infected compared to higher age groups and females. Lower age groups because of their high susceptibility and low resistance show higher infection while lower infection found in higher age groups is because of their improved immune capability which is seen to increase because of the period of exposure to infection, as has been reported by Ahmad et al.²⁶. The influence of gender on the vulnerability to infections could be attributed to the genetic predisposition and differential susceptibility owing to hormonal control. As males cover more area while grazing, this can also be the reason for their higher infection. Some studies^{23,27,28} showed similar results with respect to age groups, while others^{15,29} showed similar results with respect to sex as in the present study. However, Qamar et al.²⁸ recorded no significant difference in the percentage of infection between males and females. Biu et al.²⁷ reported 22% prevalence of gastro-intestinal (GI) parasites in males and 32% in females in case of sheep. Thus with respect to sex, it can be concluded that both sexes are equally susceptible to infection, and the difference could be the effect of management condition of host animals and may also be due to differences in the sample size.

With regard to the effect of parasite infection on haematological parameters of sheep, it was seen that there was a significant decrease in Hb concentration, PCV and RBC count in infected sheep compared to uninfected ones and the effect is seen most prominent in summer. There was a significant increase in WBC count in infected sheep compared to uninfected ones. In uninfected sheep, the decrease in Hb concentration in the summer may be due to increase in ambient temperature and haemodilution as has been reported in the literature^{30–32}. The decline in Hb concentration may also be attributed to depression of thyroid secretion, which in turn is associated with de-

creased erythropoises. Thyroid hormones have been found to increase the proliferation rate of erythroid progenitors^{33,34} and enhance the production of erythropoietic growth factors^{35,36}. However, the relatively higher values in spring may be associated with the nutritional status of the animals and climatic conditions³⁷. In case of PCV, the reduction in infected sheep may be attributed to acute loss of blood by sucking activity of H. contortus and O. ostertagi, which suck 0.05 ml blood/worm/day³⁸. In case of RBC count, it has been found that the severe anaemia may be due to chronic liver inflammation, which causes depression of erythrogenesis as has been reported in earlier studies^{8,39-43}. In case of uninfected sheep, the reduction in WBC count could be due to physiological responses to the hot climate, which also includes decrease in food intake and expansion of the plasma volume which results in haemodilution. In case of infected sheep, an increase in WBC count may be due to the immune response of the body against the parasites as a means of selfdefence. The above observations are similar to those reported in other studies with respect to PCV, RBC and WBC count⁴⁴⁻⁴⁶. In case of DLC, the results showed not much variation in uninfected sheep, but showed significant increase in neutrophils and lymphocytes in infected sheep. These results are supported by other studies^{43,47,48}. Thus there is significant association between the epidemiology and impact of these two parasites on haematology. The infection is higher in summer and so is the case with Hb, PCV and RBC count, which show significant decrease in summer compared to other seasons because of the higher prevalence in summer season. The rest of the observations also show close association between each other. Thus the present study can be useful in devising an effective control programme against these two economically important parasites.

GI parasitism represents a severe health problem in small production systems and its consequences have been found to be extensive ranging from reduced productivity to mortality. GI nematodiasis is a major threat and a primary constraint to sheep and goat productivity, as it endangers animal welfare worldwide. The main culprits are the two study parasites as they cause haemonchosis, anaemia and parasitic gastroenteritis in sheep and goats. So modern diagnostic techniques need to be incorporated to estimate the degree of infection. The present study provides information for understanding the epidemiology of H. contortus and O. ostertagi in the Kashmir Valley. It will be of potential significance in planning as well as grazing management and other prophylactic strategies for ruminants in different seasons in the Valley. Overwintering survival of these two parasites in the present study was a key factor in their epidemiology in temperate climate of Kashmir Valley. However, further experimental studies are recommended on hypobiosis which can be of help in understanding the overall physiology of these parasites.

- 1. Newton, S. E. and Munn, E. A., The development of vaccines against gastrointestinal nematode parasites, particularly *Haemon-chus contortus*. *Parasitol*. *Today*, 1999, **15**, 116–122.
- Areskog, M., Sollenberg, S., Engstrom, A., Samson-Himmelstjerna, G. and Hoglund, J., A controlled study on gastrointestinal nematodes from two Swedish cattle farms showing field evidence of ivermectin resistance. *Parasites Vectors*, 2014, 7, 13.
- Gilleard, J. S., *Haemonchus contortus* as a paradigm and model to study anthelmintic drug resistance. *Parasitology*, 2013, 140, 1506–1522.
- Jabbar, A., Campbell, A. J. D., Charles, J. A. and Gasser, R. B., First report of anthelmintic resistance in *Haemonchus contortus* in alpacas in Australia. *Parasites Vectors*, 2013, 6, 243.
- Meeusen, E. N. T., Walker, J., Peters, A., Pastoret, P. P. and Jungersen, G., Current status of veterinary vaccines. J. Clin. Microbiol. Rev., 2007, 20, 489–510.
- Soulsby, E. J. L., Genero: *Haemonchus* Cobb, 1898. In *Parasitologia yenfermedades parasitarias en los animals domesticos*. Interamericana S. A. Mexico, 1987, 7th edn., pp. 232–238.
- Benjamin, M. M., Outline of veterinary clinical pathology. Iowa State University Press Ames Iowa, USA, 1981, 2nd edn, pp. 213–238.
- Coles, E. H., Veterinary Clinical Pathology, W. B. Saunders Co. Philadelphia, USA, 1986, IV edn.
- Coffin, D. L., Manual of Veterinary and Clinical Pathology, Comstock Publishing Associates Inc. Ithaca, New York, USA, 1995, 3rd edn.
- 10. England, J. M. and Bain, B. J., Total and differential leukocytes counts. Br. J. Haematol., 1976, 33, 1.
- Marshal, P. N., Bentley, S. A. and Levis, S. M., An evaluation of some Romanowsky stains prepared from some purified dyes. *J. Clin. Pathol.*, 1975, 28, 680.
- Conn, H. J. and Darrow, M. S., Staining procedure used by the Biological Staining Commission, Williams and Wilkins Company, Baltimore, 1960, 2nd edn.
- Simmons, A., Leverton, P. and Elbert, G., Normal laboratory values for differential white cell counts established by manual and automated cytochemical methods (Hemalog D). J. Clin. Pathol., 1974, 27, 55.
- Schalm, O. W., Jain, W. C. and Carrol, E. J., Veterinary Haematology, Lea and Febieger, Philadelphia, USA, 1975, 3rd edn, pp. 1–807.
- Tasawar, Z., Naz, F., Lashari, M. H. and Hayat, C. S., Prevalence of *Ostertagia* spp. in a sheep herd. *Sarhad J. Agric.*, 2011, 27, 275–278.
- Kumsa, B. and Wossene, A., Abomasal nematodes of small ruminants of Ogaden region, eastern Ethiopia: prevalence, worm burden and species composition. *Rev. Med. Vet.*, 2006, **157**, 27–32.
- Khan, M. N., Sajid, M. S., Khan, M. K., Iqbal, Z. and Hussain, A., Gastrointestinal helminthiasis: prevalence and associated determinants in domestic ruminants of district Toba Tek Singh, Punjab, Pakistan. *Parasitol. Res.*, 2010, **107**, 787–794.
- Tariq, K. A., Chishti, M. Z., Ahmad, F. and Shawl, A. S., Anthelmintic activity of extracts of *Artemisia absinthium* against ovine nematodes. *Vet. Parasitol.*, 2009, 160, 83–88.
- Lone, B. A., Chishti, M. Z., Ahmad, F. and Tak, H., A Survey of gastrointestinal helminth parasites of slaughtered sheep and goats in Ganderbal, Kashmir. *Global Vet.*, 2012, 8, 338–341.
- Shwakat, M. E., Awad, F. I., Abdel-Halin, M. M., Elsayed, M. and Afify, M., Studies on the incidence and changes associated with abnormal nematodes in sheep. *Egypt. J. Comp. Pathol. Clin. Pathol.*, 1994, 7, 25–33.
- Javed, M. S., Iqbal, Z. and Hayat, B., Prevalence and economics of haemonchosis in sheep and goats. *Pak. Vet. J.*, 1992, 12, 36–38.

- Makhdoomi, D. M., Nasreen, S., Banday, S. D. and Moulvi, B. A., Incidence of different bovine gastrointestinal parasites in Kashmir. *Indian Vet. J.*, 1995, **72**, 898–900.
- Lateef, M., Iqbal, Z., Jabbar, A., Khan, M. N. and Akhtar, M. S., Epidemiology of trichostrongylid nematode infections in sheep under traditional husbandry system in Pakistan. *Int. J. Agric. Biol.*, 2005, 7, 596–600.
- Jabeen, F., Ahmad, N., Ahmad, K. M., Chaudhry, M. A. and Ali, S., Studies on the epidemiology and chemotherapy of haemonchosis in sheep in the Punjab. *Pak. Vet. J.*, 2000, 20, 90–92.
- Kates, K. C., Survival on pasture of free living stages of some common Gastrointestinal nematodes of sheep. *Proc. Helminthol. Soc. Wash.*, 1950, 17, 39–58.
- Ahmad, F., Tariq, K. A. and Chishti, M. Z., Epidemiological studies on haemonchosis of sheep in Kashmir Valley. *Orient. Sci.*, 2007, 12, 43–47.
- 27. Biu, A. A., Maimunatu, A., Salamatu, A. F. and Agbadu, E. T., A faecal survey of gastrointestinal parasites of ruminants on the University of Maiduguri Research Farm. *Int. J. Biomed. Health Sci.*, 2009, **5**, 175–179.
- Qamar, M. F., Maqbool, A., Khan, M. S., Ahmad, N. and Muneer, M. A., Epidemiology of *Haemonchosis* in sheep and goats under different managemental conditions. *Veter. World*, 2009, 2, 413– 417.
- Gorski, P., Niznikowski, R., Strzelec, E., Popielarczyk, D., Gajewska, A. and Wedrychowicz, H., Prevalence of protozoan and helminth internal parasite infections in goat and sheep flocks in Poland. *Arch. Tierz. Dummerstorf.*, 2004, 47, 43–49.
- Appleman, R. O. and Delouche, J. C., Behavioural, physiological and biochemical responses of goats to temperature 0° to 40°C. *J. Anim. Sci.*, 1958, 17, 326–335.
- Bianca, W., Thermoregulation. In *Adaptation of Domestic Animals* (ed. Hafez, S. E. S.), Lea and Febiger, Philadelphia, USA, 1968.
- Prasetyo, S., Miller, H. M., Scheurman, E. A. and Holmes, J. H. G., The effect of heat and humidity on pregnant feral goats. *Animal Prod.*, 1984, 15, 541–544.
- Popovic, W. J., Brown, J. E. and Adamson, J. W., The influence of thyroid hormones on *in vitro* erythropoiesis. *J. Clin. Invest.*, 1977, 60, 907–913.
- Dainiak, N., Hoffman, R., Maffei, L. A. and Forget, B. G., Potentiation of human erythropoiesis in vitro by thyroid hormone. *Nature*, 1978, 272, 260–262.
- Dainiak, N., Sutter, D. and Kreczko, S., The blood picture L-triiodothyronine augments erythropoietic growth factor release from blood and bone marrow leukoyctes. *Blood*, 1986, 68, 1289– 1297.
- Fandrey, J., Pagel, H., Frede, S., Wolff, M. and Jelkmann, W., Thyroid hormones enhance hypoxia induced erythropoietin production *in vitro*. *Exp. Haematol.*, 1994, 22, 272–277.
- Payne, W. J. A., An Introduction to Animal Husbandry in the Tropics, Longman Scientific and Technical, England, UK, 1990.
- Soulsby, E. J. L., Helminth, Arthropods and Protozoa of Domesticated Animals, Bailler, Tindall, London, 1982, 7th edn, pp. 212– 242.
- Kaneko, J. J., Harvey, J. W. and Bruss, M. L., *Clinical Biochemistry of Domestic Animals*, Academic Press, San Diego, USA, 1997, 5th edn, pp. 890–894.
- 40. Kramer, J. W., Normal hematology of cattle, sheep and goats. In Schalm's veterinary hematology (eds Feldman, B. F., Zinkl, J. G. and Jain, N. C.), Lippincott Williams & Wilkins, Philadelphia, 2000, 5th edn, pp. 1075–1084.
- 41. El-Sayed, M., El-Ramady, R. A. and Tawfik, S. A., Effect of mirazid on fascioliasis and blood chemistry in sheep. *Egyptian J. Agric. Res.*, 2003, **81**, 785–796.
- Lotfy, H. S., Mahmoud, S. M. and Abdel-Gawad, M. A., Some studies on fascioliasis in mid-Egypt. Agric. Res., 2003, 81, 209– 227.

CURRENT SCIENCE, VOL. 113, NO. 9, 10 NOVEMBER 2017

- 43. Omran, H. H. and El-Kholony, K. H., The influence of Nitroxynil on clinical, biochemical and hematological parameters in Egyptian buffaloes affected with liver flukes in Sharkia goveronerate. *Egyptian J. Agric. Res.*, 2003, **81**, 739–751.
- Mottelib, A. A., Haroon, E. M., Magzoob, M. and El-Basheer, E., The effect of gastrointestinal parasites on the blood picture of sheep and goats at Al-Gassim. *Assiut Vet. Med. J.*, 1992, 28, 215– 223.
- 45. Esmaeilnejad, B., Tavassoli, M. and Asri-Rezaei, S., Investigation of hematological and biochemical parameters in small ruminants naturally infected with *Babesia ovis. Vet. Res. Forum*, 2012, **3**, 31–36.
- El-Aziz, M. Z. A., Emara, S. A. and Salem, F. S., Clinicopathological studies on fascioliasis among sheep in Giza province. *Egypt. J. Veter. Sci.*, 2002, 36, 75–86.
- 47. Waweru, J. G., Kanyari, P. W. N., Mwangi, D. M., Ngatia, T. A. and Nansen, P., Comparative parasitological and hematological changes in two breeds of sheep infected with *Fasciola gigantica*. *Trop. Anim. Health Prod.*, 1999, **31**, 363–372.
- 48. Zhang, Z. W. Y., Moreau, E., Hope, J. C., Howard, C. J., Huang, W. Y. and Chauvin, A., *Fasciola hepatica* and *Fasciola gigantica*: comparison of cellular response to experimental infection in sheep. *Exp. Parasitol.*, 2005, **111**, 154–159.

ACKNOWLEDGEMENT. We thank the Director CORD, University of Kashmir, Srinagar for providing the necessary facilities during this study.

Received 22 April 2016; revised accepted 18 May 2017

doi: 10.18520/cs/v113/i09/1776-1783

Isolation of *Listeria monocytogenes* from peridomestic birds and captive wild animals

U. V. Jagtap¹, R. P. Kolhe^{1,*}, P. D. Deshpande¹, N. V. Kurkure², C. V. Dhandore¹, D. M. Muglikar³, S. N. Jadhav⁴, N. K. Nighot⁵ and S. B. Barbuddhe⁶

¹Department of Veterinary Public Health,
³Department of Veterinary Microbiology, and
⁴Department of Veterinary Biochemistry,
Krantisinh Nana Patil College of Veterinary Sciences,
Shirwal 412 801, India
²Department of Veterinary Pathology, Nagpur Veterinary College,
Seminary Hills, Nagpur 440 006, India
⁵Rajiv Gandhi Zoological Park and Wildlife Research Center, Katraj,
Pune 411 037, India
⁶ICAR-National Institute of Biotic Stress Management,
Raipur 493 225, India

Listeria monocytogenes is an important foodborne pathogen responsible for septicaemia, meningitis and abortions. There are several animal reservoirs; however, the role of wild animals and peridomestic birds remains underestimated. We have screened 270 faecal samples of wild animals in captivity (18 species) and peridomestic birds (12 species). *Listeria* species were isolated from seven (6.66%) mammals and two (1.21%) birds. *L. monocytogenes* was isolated from barking deer, porcupine, pigeon and crow. Isolated *L. monocytogenes* were virulent strains of 4b serogroup. There is a need to explore the role of such non-conventional sources in the spread of *L. monocytogenes* in nature.

Keywords: Antibiotic sensitivity, birds, *Listeria monocytogenes*, serotyping, wild animals.

LISTERIA monocytogenes is an emerging foodborne pathogen recognized globally. Ecology of *L. monocytogenes* is atypical and has several animal and inanimate reservoirs in nature¹. Listeria infection is mainly acquired through a variety of contaminated non-meat and meat foods. Manifestations of listeriosis both in animals and humans include septicaemia, meningitis, abortion and still birth. Several virulence factors encoded by the *hlyA*, *plcA*, *actA* and *iap* genes play a significant role in the pathogenesis of *L. monocytogenes* infections².

Even though several studies have demonstrated broader distribution of Listeria species throughout the natural environment, the role of wild animals and birds in the ecology and as reservoirs of L. monocytogenes is not clearly understood³. Limited studies on the detection of pathogenic bacteria including L. monocytogenes from wild animals and birds have been documented^{4–7}. Studies on the detection of Listeria from sources other than foodprocessing environments may help reveal the population genetics and natural history of *Listeria* species⁸. Due to several anthropogenic consequences, emerging trends in the epidemiology of infectious diseases have been recorded⁹. Living in close proximity and sharing the same environment virtually creates zoonotic nidus. Thus, asymptomatic healthy carriers are the cause of concern. In India, adequate studies on the occurrence of L. monocytogenes from foods have been conducted^{5,10,11}; however, studies on wild animals and birds are largely lacking. Listeria species may survive for a longer period in the soil and possibly get excreted in the faeces of carrier animals without any symptoms, and may cause infection to other animals as well as personnel working in the zoo and visitors. The present study was conducted to isolate L. monocytogenes in peridomestic birds and wild animals in captivity.

Fresh faecal samples (n = 270) comprising 105 from mammals and 165 from peridomestic birds were collected. Samples were collected from the nesting sites of birds (12 species) with sterile swab without touching or disturbing their habitat. Samples of wild animals (18 mammals) were collected from the Rajiv Gandhi Zoological Park and Wild Life Research Centre, Katraj, Pune, India. The birds included pigeon (n = 80), sparrow

^{*}For correspondence. (e-mail: dr_kolherahul@rediffmail.com)

CURRENT SCIENCE, VOL. 113, NO. 9, 10 NOVEMBER 2017