MATERIALS AND METHODS

External jugular vein blood samples were collected in potassium ethylene diamine tetraacetate (K_3EDTA) vacuum tubes (Becton-Dickinson vacutainer) from 72 West African dwarf goats of which 36 (group 1) had been infected with 1000 metacercaria of *Schistosoma bovis* subcutaneously six weeks before. The rest were uninfected controls (group 2). The number of erythrocytes was determined in a Coulter counter model ZF, while haemoglobin concentration (Hb) and total leukocyte counts were determined in model S560 Coulter counter. Haematocrit was determined in microhaematocrit capillary tubes at 12,000 G in a microhaematocrit centrifuge (CLAY-ADAMS).

Mean corpuscular volumes (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) were calculated according to standard formulae. Basophils, eosinophils, monocytes, lymphocytes, band and segmented neutrophils were counted out of 200 cells total on thin blood films stained with Romanowsky stain and mounted under coverslips with xylene.

The results were statistically analyzed with Statistical Analysis System (SAS software, 1988). Means and logarithmic means of parameter values of infected and control goats were compared by t test and general linear models. Frequency distribution curves of the logarithms of parameters of infected and control goats were prepared. Logarithmic frequency distributions accurately describe the biological systems (Flensburg and Willeberg, 1976). The two distribution curves had different peaks at their respective arithmetic means with their neighbouring tails overlapping. Critical values were selected in these overlapping zones. These critical values were used in deciding whether the alteration in the blood parameter was so low or high. Animals with larger alterations than the critical values of specific parameters were test positives (T+) and those within were negatives (T-). The sensitivity and specificity of each test were calculated (Martin *et al.*, 1987). Other goats of Danish landrace breed comprising of 40, 6 - 10 months of age (group 3), 80 pregnant adults (group 4), 43, 78 and 63 adults at 20 - 30 (group 5), 60 - 90 (group 6) and more than 120 (group 7) days in lactation, all free from Schistosomiasis were similarly examined. Group 1 and 2 were identical except the infectious status, 1 - 3 differed with regard to infection and breed only. Group 2 and 3 were identical except for breed, 3, 4, 5, 6 and 7 differed only in age and state of physiology. Groups 4, 5, 6 and 7 differed only for the physiological states. The proportions of T+ goats in group 3 - 7 were determined at the same critical values.

RESULTS

The number of red blood cells (RBC), haemoglobin concentration, packed cell volume (PCV), MCV, MCH and lymphocyte count were significantly lower in infected than in control goats (Table 1). MCHC and monocyte count did not differ significantly in the two groups. Total white blood cells (WBC), absolute number of basophils, eosinophils, band and segmented neutrophils were higher in infected than in control goats (Table 1). Up to 30.3 $x10^9$/l leukocytes were observed compared with a maximum of $23.6x10^9$/l in control goats. An erythrocyte count of $13.55x10^{12}$/l ($\log_{10}$ = 1.13) was critically low for dwarf goats at 6 - 10 months of age, with a mean of around 16 - $18x10^{12}$/l erythrocytes. Those with RBC counts below this were T+ for anemia (Table 2-3). The critical values for PCV, Hb and lymphocyte count were 0.30 (log=-0.52), 6.60 mmol/l (log Hb 0.819) and $6.0x10^9$/l ($\log_{10}$ = 0.778) respectively, whereas goats with lower values than these were T+. The critical value for WBC was 18.5, basophils 0.15, band neutrophils 0.35,

Table 1: The mean ± s.d. of blood parameters in infected and noninfected goats.

Parameter	Infected	Control	SE	SP
RBC $x10^{12}/l$	11.52±2.41	14.63±1.49***	77.8	80.6
PCV l/l	0.25±0.063	0.35±0.056***	69.4	88.9
Hb mmol/l	5.54±1.50	7.44±1.06***	72.2	75.0
MCV fl	22.20±2.51	23.91±3.58*		
MCH fmol	0.48±0.068	0.51±0.055*	44.4	80.6
MCHC mmol/l	21.45±2.47	21.6±3.33^{NS}		
WBC $x10^9/l$	18.80±4.77	16.25±3.36***	47.2	80.6
Basophil $x10^9/l$	0.46±0.33	0.14±0.15***	75.5	83
Monocyte $x10^9/l$	0.58±0.50	0.39±0.33^{NS}		
Eosinophil "	0.69±0.67	0.19±0.29***	61.1	91.2
Lymphocyte "	6.54±2.12	8.58±2.65***	44.4	75.0
Bands "	0.81±0.75	0.16±0.17***	63.9	83.3
Segmenter "	9.69±4.44	6.80±2.82**	61.1	72.2

***p<0.0001, **p<0.001, *p<0.05, SE= sensitivity, SP= specificity; critical values in materials and methods.

Table 2. Proportions of T+ 6 - 10 months old infected (group 1) and control dwarf (group 2) goats, and disease free landrace goats 6 - 10 months old (group 3) and over two years adult; pregnant (group 4), early lactating (group 5) mid lactating (group 6) and late lactating landrace goats (group 7) at the same condition values.

Group	1	2	3	4	5	6	7
(n)	36	36	40	80	43	78	63
RBC	0.78	0.19	0.83	0.99	1.00	0.97	0.86
Hb	0.72	0.25	0.40	0.50	0.28	0.52	0.59
PCV	0.69	0.11	0.45	0.38	0.44	0.51	0.54
Band	0.64	0.17	0.08	0.19	0.49	0.36	0.41
Baso	0.75	0.42	0.00	0.05	0.12	0.05	0.05
Eos	0.61	0.08	0.15	0.18	0.23	0.51	0.40
WBC	0.47	0.19	0.10	0.03	0.19	0.13	0.08
Lymph	0.44	0.25	0.35	0.56	0.46	0.68	0.60
Segm	0.61	0.28	0.05	0.09	0.42	0.35	0.30

Baso = basophils, lymph = lymphocytes, Segm = segmented neutrophils, high T+ propotions observed for infected goats of group 1.

segmented neutrophils 8.00, eosinophils 0.38×10^9/l or their logarithms at 1.267, -0.824, -0.456, 0.903 and -0.420 respectively. Animals with values above these were T+.

Twenty two of the 36 infected goats were T+ to series interpretation of PCV, RBC and Hb tests compared with only two of the controls. In basophilia, eosinophilia and band neutrophilia tests 12 infected goats were T+ compared with one control. For basophilia and eosinophilia 18 infected and two control goats were T+ and eosinophilia and lymphopaenia tests resulted in 25 % sensitivity and 94.4 % specificity.

Erythrocyte absolute indices were only slightly affected by the infection. MCV, MCH and MCHC in infected goats (values for controls in parentheses) were 22.2 ± 2.51 fl (23.9 ± 3.58), 0.48 ± 0.068 (0.51 ± 0.055) fmol and 21.45 ± 2.47 (21.60 ± 3.33) mmol/l. MCV, MCH, MCHC and monocyte counts were excluded in the test parameters on account of the very slight differences between infected and control animals.

The tests on the clinically healthy group 3 - 7 landrace goats showed a variable number of false T+ animals. Some parameters were influenced by age, breed and lactation. Nevertheless many infected dwarf and landrace disease free goats had RBC counts below 13.55×10^{12}/l. There was also a variation among the different physiological groups. Group 4 - 6 had lower RBC counts than others, thus more were false T+ with respect to this parameter. Group 3 landraces had lower RBC counts than group 2 control dwarf goats of the same age, thus many false T+ with respect to anaemia. Similar patterns were observed for Hb and PCV. PCV and Hb were better estimates for anemia than absolute RBC count.

Clinical haematology on healthy 6 - 10 months old, pregnant, 20-30, 60-90 and more than 120 days postparturient adult landrace goats showed a variable number of T+ goats with respect to leukocyte counts, evidently due to an effect of age, breed and state of physiology. The majority of goats were T- to basophilia, eosinophilia, neutrophilia and leukocytosis while many infected goats were T+, thus distinctly identifiable from the disease free animals. Both control dwarf and disease free landrace goats of similar age (groups 2 and 3) responded equally to the leukocyte count tests. Most were T- as opposed to many T+ in the infected group, indicating that breed was not a major altering factor. In the adults, many pregnant goats were false T+ to lymphopaenia. Many false T+ to neutrophilia, eosinophilia and lymphopaenia were observed in groups 5, 6 and 7 (all lactating).

Sensitivity interpretations of clinical haematological tests in goats infected with *Schistosoma bovis* revealed sensitivities of 77.8, 69.4, 72.2, 47.2, 75.0, 61.1, 61.1 and 44.4 % for decreased number of erythrocytes, lowered haematocrit, haemoglobin concentration, leukocytosis, basophilia, neutrophilia, eosinophilia and lymphopaenia respectively. Series interpretation of RBC, PCV and Hb as well as of basophilia and eosinophilia gave sensitivity of 61.1 % and 50 % respectively. The respective specificities were 80.6, 88.9, 75.0, 80.6, 58.3, 72.2, 91.7 and 75.0 % and 94.4 % for both series interpretations. Haematological tests depend on both careful selection of healthy reference group values and final interpretations.

DISCUSSION

Passage of worm eggs through walls of intestine in schistosomiasis leads to haemorrhagic enteritis (Saad *et al.*, 1984a; Vercruysse *et al.*, 1988), with subsequent decrease in erythrocyte counts, Hb and PCV, which were observed in present infected goats. The anemia was normocytic and normochromic because MCV, MCH and

MCHC were not significantly altered. Similar observations are reported in cattle, sheep and goats (Monrad *et al.*, 1982; Saad *et al.*, 1984a).

The sensitivities of RBC, Hb and PCV, as test parameters for detection of Schistosomiasis were in the range of 69.4 % to 77.8 %. Twenty eight of the 36 infected animals were identified by the RBC test at a critical value of $13.55x10^{12}/l$ compared with 7 in the control group. Twenty six and 25 were T+ to Hb and PCV respectively in infected goats against 9 and 4 for the control goats. Assessment of Hb and PCV gives a conclusive evidence of any existing anaemia, justifying their use for diagnosis of parasitism (Paries *et al.*, 1982; Kalu and Lawani, 1986). These tests are however surrogative because they measure the effects of parasitism, rather than detecting the parasite itself. The present results are also based on point estimates of the day of sampling. Different results could be obtained on a different day of sampling.
The false T- goats to RBC, Hb and PCV in the infected group were probably caused by the fact that not all infected goats were severely affected, especially because blood sampling at 6 weeks post infection was too early for extensive tissue damage, or due to individual animal variations. The specificities were high in RBC, PCV and Hb. A series interpretation for RBC, Hb and PCV indicated moderately high sensitivity and specificity greater than in parallel interpretation of individual parameters. Combining tests (series interpretation) gives less chance for an animal to react to all of them. Sensitivity is decreased while specificity is increased. A parallel interpretation gives sick animials the widest chances to react since an animal is taken to be T+ (therefore sick) if it reacts to either of the tests.

The increase of total leukocyte counts, band and segmented neutrophils, basophils and eosinophils and the lymphopaenia in infected goats have been observed also by Massoud (1973), Kassuku *et al.* (1986), Vercruysse *et al.* (1988). Similar results have been found in sheep (Monrad *et al.*, 1982; Vercruysse and Schanderyl, 1986; Vercruysse *et al.*, 1985, 1988), cattle (Saad *et al.*, 1984a; b) and pigs (Yason and Novilla, 1984). Each leukocyte type is produced and distributed independently of the others therefore absolute numbers were used for comparison purposes.

Neutrophilia was evidently due to inflammatory reactions in the gastrointestinal tract and liver, while lymphopaenia could be due to damaged lymph nodes (Saad *et al.*, 1984a) or migration to tissues where lymphocytes infiltrate perivascular spaces such as calcified foci, fibrous scars, granulomas, parasites and eggs in the liver as well as lymph nodes and intestinal mucosa. Eosinophils infiltrate worms and parasite eggs in hepatic and gastrointestinal sites (Saad *et al.*, 1984a). Eosinophilia in parasitism is due to helminthic antigens. The basophil granule chondroitin and damatan sulphates, heparin, histamine, acid/alkaline phosphatases, acid hydrolysases, peroxidases and serotonin increase in nephrosis, hepatopathies, hyperlipoproteinemia and high IgE production, all known to occur in parasitism.

The sensitivities of basophilia, neutrophilia and eosinophilia were high (> 60 %). The specificities were high for eosinophilia, neutrophilia, leukocytosis and lymphopaenia but low for basophilia. False T- were probably due to variations in leukocyte responses to the disease in individual animals. When the tests were interpreted in a serial manner for basophilia, eosinophilia and neutrophilia (bands) 12 infected goats and one control were T+. For basophilia and eosinophilia, 18 infected and 2 control goats were T+, the sensitivity and specificity being 50 and 94.4 % respectively. Eosinophilia and lymphopaenia had 25 % sensitivity and 94.4 % specificity. The selection of series or parallel interpretation depends on the purpose of the test. A

subclinical disease such as parasitism for treatment purposes demands a high sensitivity test, whereas a disease for eradication requires a highly specific test. Nielsen *et al.* (1985) examined foecal samples of goats from an area with high parasite infections. The results of the foecal samples were consistently negative but examination of leukocyte counts revealed eosinophilia and lymphopaenia. This finding indicates that if carefully interpretated clinical haematological tests are better than foecal tests.

Differences in values of blood parameters due to age, breed, physiological states and season have been observed in many animals (Shaffer *et al.*, 1981; Domina *et al.*, 1982: Wilson *et al.*, 1986; Somvanshi *et al.*, 1987) therefore clinical haematological tests are likely to point out many animals reacting as false T- and T+. A high proportion of false T+ to the RBC test were observed in group 3 - 7 goats at the same critical values. Since group 3 goats were of the same age as the infected and control dwarf goats, this appears to be a breed rather than an age influence. Similar results were obtained for Hb and PCV. The differences in proportions of the T+ for the adult goats varied in the four groups 4 - 7 indicating a lactation influence. The differences between group 3 and 4-7 are probably due to age. Many of the infected goats were T+ to neutrophilia, basophilia, eosinophilia, leukocytosis, lymphopaenia and neutrophilia but control dwarf goats (group 2) and disease free landrance goats of the same age (group 3) showed identical results except for a slightly higher proportion of false T+ for lymphopaenia in the latter than in the former. The breed influence was thus not significant in leukocyte counts. High proportions of group 4-7 goats were false T+ to lymphopaenia, 5 and 7 to band neutrophilia, 5, 6 and 7 to segmented neutrophilia and 6 and 7 to eosinophilia indicating an influence of age and lactation / pregnancy. The variations within groups 4-7 were due to lactation or pregnancy whereas those between on one hand groups 4 - 7 and on the other 2 and 3 were due to age.

The leukocytosis with neutrophilia in group 5 goats was probably due to the physiological leukocytosis often seen at parturition and estrus, in response to high chances of bacterial infections associated with these events (Oltner and Berglund, 1983). This might explain why some animals were false T+ to leukocyte tests. The number of T+ to leukocytosis falls with increasing postparturient period and has been observed to decline with lactation also in Baladi goats (Hassan *et al.*, 1986).

In conclusion clinical haematologic tests involving leukocytosis, eosinophilia, basophilia, neutrophilia, lymphopaenia, lowered PCV, Hb, and erythrocyte number show high sensitivities and specificities for diagnosis of parasitic infections if carefully interpreted. The influence by age, state of physiology and breed must be controlled by proper selection of critical values in relation to the reference range of the level of parameters in healthy animals.

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