HAEMATOLOGY-BASED PREDICTION OF LESION STATUS IN BOVINE TUBERCULOSIS OF FARMED RED DEER

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We have been investigating the use of haematological profiles to identify the degree of severity of lesions in herds known to have a bovine tuberculosis problem. The purpose of this paper is to review progress to date.

TECHNIQUE DEVELOPMENT

Choice of techniques was governed by the need for a high degree of precision - to provide maximum diagnostic potential - and the need for automated techniques, or techniques capable of automation, in view of the large numbers of specimens to be processed.

Inflammatory cells. Many years ago, Rumke drew attention to the considerable uncertainty in manual differential leucocyte counts on 100 cells, even assuming perfect technique and random distribution of cells. The first requires considerable skill and the second is impossible to achieve with the commonly used 'spreader slide' method. Extending the count to 200 cells does not greatly improve the precision.
In an automated differential leucocyte count system such as the Technicon H6000/C, 8-10,000 cells are counted, and the cells are randomly distributed. The precision is therefore considerably better (Fig. 1). We have so far processed more than 10,000 deer samples through such a system. Results show excellent agreement with manual methods (Table 1). A more complete discussion of the application of this technology to deer blood is in preparation. There are two drawbacks in the context of this work - monocytes are not detected, and juvenile neutrophils are not detected as such, although the 'high peroxidase' cells offer a useful alternative indicator.

Inflammatory Proteins. The options here are numerous, and have recently been reviewed for Rheumatoid arthritis (Bull et al. 1986). Two techniques have been used regularly in this study, fibrinogen estimation and plasma viscosity. We use a thrombin clotting time for fibrinogens, with a BBL fibrometer (BBL, Cockeysville, Maryland, USA). This has been calibrated against the so-called 'Aberdeen method' (fig. 2) which has been shown to

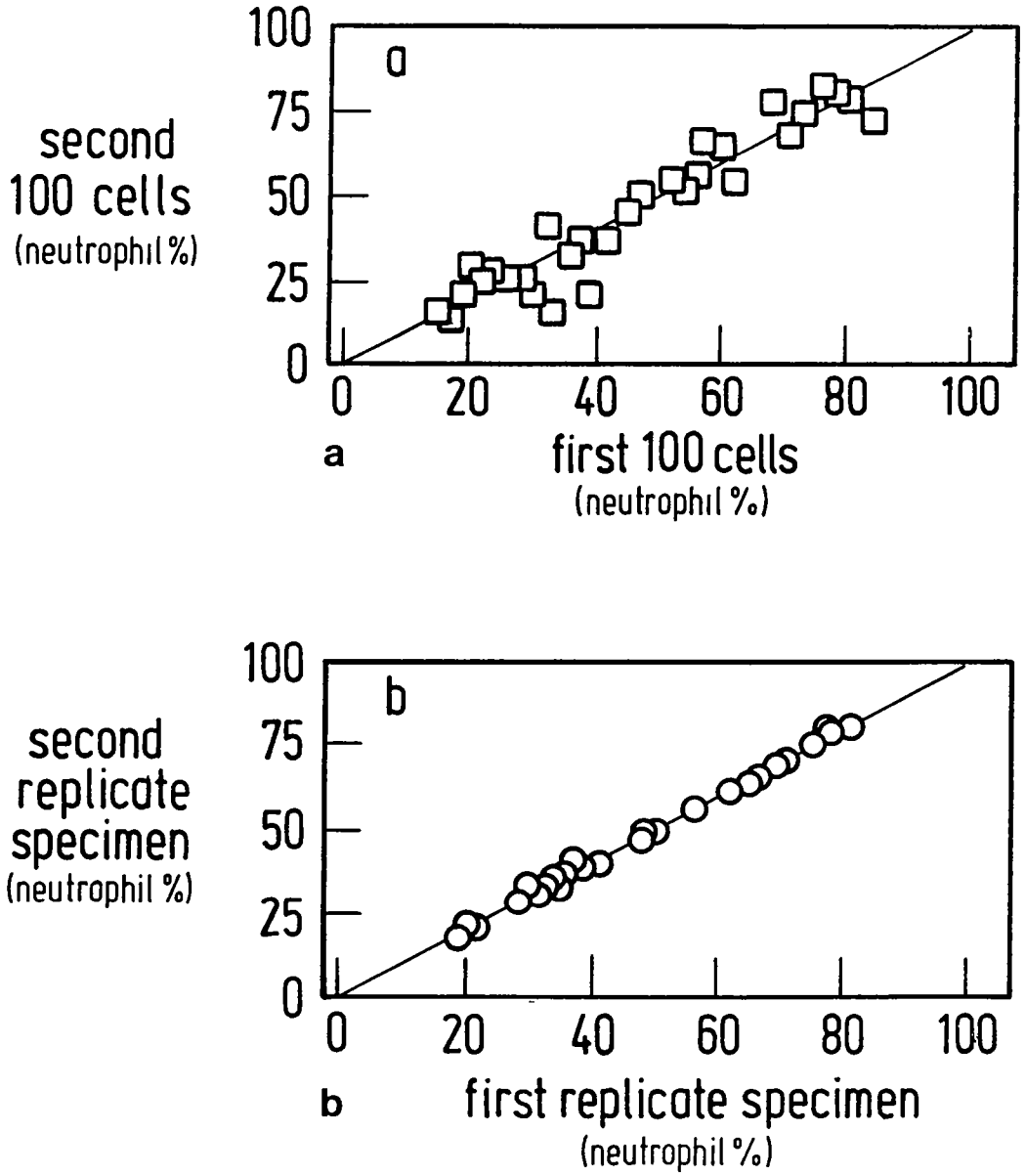

Fig.1: Precision of differential counts on deer blood neutrophils performed using a) manual method, b) Technicon H6000.

1a: Manual differential count - scatter seen when plotting neutrophil percentages from 2 successive 100 cell differentials (same specimen).

1b: Technicon H6000 - scatter seen when plotting neutrophil percentages from two aliquots of the same specimen.

CELL TYPE	MEAN x 10^9/L MANUAL	AUTOMATED	PEARSONS R
Neutrophil	3.21	3.49	0.996
Lymphocyte	3.05	2.93	0.970
Eosinophil	0.175	0.17	0.94

TABLE 1: Comparison of manual and automated (H6000/C) differential leucocyte counts on red deer. (N = 87).

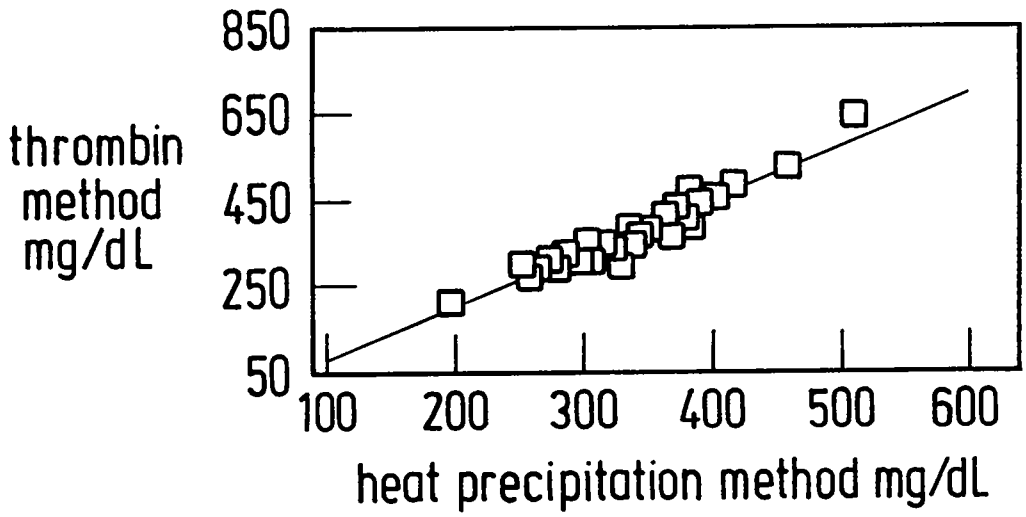

FIG 2: Comparison of thrombin clotting time and heat precipitation methods for fibrinogen estimation in EDTA plasma from red deer.

PARAMETER	Min.	Max.	UNITS	R	t	PROBABILITY
Fibrinogen	269	1200	mg/dL	0.83	8.069	<0.001
alpha-1-glob.	2.14	5.63	g/L	0.27	1.54	0.13
alpha-2-glob.	3.11	19.4	g/L	0.83	8.02	<0.001
beta-glob.	1.31	11.6	g/L	-0.05	-0.298	<0.765
gamma-glob.	12.5	35.7	g/L	0.58	3.90	<0.001

TABLE 2: Correlation of plasma viscosity with plasma protein fractions.

be both specific and accurate, except in the case of paraproteinaemias. (Millar, Stimpson and Stalker 1971, Dintenfass and Kammer 1976). The thrombin kit used in this work compares very well with the Aberdeen method (Pearson R = 0.991 for n = 45), although it should be noted that some other commercial kits have been found to be unreliable for deer plasma collected into EDTA (personal observation).

Plasma viscosity has been little used in deer, but has been used in tuberculosis in humans (Bircher, cited by Harkness, 1971). It is a robust technique in that good results can be obtained on samples several days after specimen collection, provided the plasma is separated within 24 hours, and storage is at room temperature. Essentially the technique consists of pushing plasma through a capillary tube in closely regulated conditions of pressure and temperature, and finding how long a set volume takes to pass through the capillary. Table 2 shows the effect on plasma viscosity of various plasma proteins in deer. The results are very similar to those found in humans, as reviewed by Harkness (1971).
For the purpose of judging the efficacy of predicting lesion severity, the following scales were used:

RISK ASSESSMENT	LESION SCORE
0 = zero risk	0 = NVL
1 = low	1 = enlarged lymph nodes only
2 = moderate	2 = calcified lesions
3 = fairly high	3 = caseous lesions
4 = high	4 = single small liquefactive lesion
5 = virtually certain	5 = 2 or more small liquefactive, 1 or more moderate size.
	6 = 1 large liquefactive, or GTB with no liquefactive lesions.
	7 = Multiple lesions, generalised Tb.

RESULTS

With the invaluable help of a number of veterinary practitioners, pilot studies using the above techniques were carried out last year (table 3).

Farm 1 Lesion grade	Fibr'gen mg/dL	PV cp	Hb g/L	%Neutr.	NAN x10^9/L	n
4 to 7	373	1.27	177.5	70.1	4.57	14
1 to 3	351	1.23	177.3	58.7	3.38	17
0	274	1.18	183	65.9	3.43	20
Farm 2						
4 to 7	326	1.20	170.2	73.8	4.84	35
0	220	1.09	175	57.9	2.71	22

TABLE 3: Mean values for some haematological parameters in two herds

Reference values were prepared on non-tuberculous deer using the same technology, and statistical techniques discussed elsewhere (Cross, 1983). These provide the upper (95%) limits used for table 4.

Lesion grade	Fibr'gen mg/dL >350	PV cp >1.20	%Neutr. >61%	NAN x10^9/L >4.14
4 to 7	17/31 (55%)	23/32 (72%)	35/40 (88%)	27/40 (68%)
1 to 3	9/26 (35%)	8/20 (40%)	14/22 (64%)	11/22 (50%)
0	5/40 (13%)	7/34 (21%)	23/55 (42%)	10/55 (18%)

TABLE 4: Proportion of abnormally high results compared to lesion severity in two Tb. herds

The above data was prepared from the blood test result obtained nearest to the date of post-mortem. Inspection of table 4 indicates that there is a greater preponderance of high results among the animals with more severe lesions. No single test in isolation offers a clear indication of lesion severity. However, by considering the combination of tests and particularly changes in profiles over time, retrospective predictions were attempted on 89 'at risk' animals, for which a reasonable amount of data was available. The results are shown in table 5:

Lesion status	Risk Assessment						
	0	1	2	3	4	5	Total
0	30	7	1*	3*	1*		42
1			1				1
2	1	4		2*			7
3	1	6	2	1			10
4					1		1
5			2	3	2	1	8
6			1*	1	1		3
7			1*	4*	9	3	17

* Risk assessments not considered acceptable following post mortem.

TABLE 5: Predictions of lesion status in deer from bovine Tb. herds

Thus predictions on lesion status were successful in 76 animals (85%). All animals with liquefactive lesions or generalised Tb. were rated at least moderate risk, with 25 of these rated as at least 'fairly high risk'. At the other end of the scale, 30 (71%) of those with NVL were rated as zero risk, with a further 7 rated low risk. One animal with multiple lesions was among the 'moderate risk' group. In this case, the last specimen was taken 5 weeks before post mortem, and showed a distinctly raised neutrophil count. Had a further specimen been obtained closer to the time of post-mortem, it seems likely that the true inflammatory status of this animal would have been disclosed.
The high risk animal that was found to have no lesions consistently had a raised fibrinogen, borderline or slightly increased plasma viscosity, slightly raised neutrophils and a suspiciously low haemoglobin. There was no obvious explanation for these results. As the animal had a negative skin test and negative lymphocyte transformation, the haematology would not have been an important consideration from the tuberculosis point of view

if the herd had not been an experimental one.

We have recently confirmed an earlier suspicion that the application of a skin test can alter the results of inflammatory status testing quite substantially (Cross, Mackintosh and Griffin, in preparation). Failure to appreciate this may well have caused confusion in interpreting earlier data. Animals which have shown very modest lymphocyte transformation and no haematological signs of inflammation may develop quite abnormal signs following a skin test. This is illustrated in fig. 3 (radial plot diagrams after Love, 1984). This animal appeared substantially normal on each of 4 tests over a 4 month period. At the time of reading the skin test there were clear haematological signs that an inflammatory process was in progress. The profile returned to normal quite slowly, and had still not returned to pre-skin test values 9 days later. Following the accidental death of this hind, no lesions were detected at post-mortem examination. This post-skin test phenomenon has been observed in 'at risk' herds only to date. A control of 24 healthy animals not at risk has shown no such effect. There is no obvious cause, and the phenomenon is currently under investigation.

SAMPLING

Haematology profiles are clearly non-specific, and a single specimen is unlikely to be helpful in establishing lesion status in 'at risk' herds. A minimum approach would be 2 specimens taken 3-4 weeks apart, which would allow division into those requiring further investigation, and those in which the picture is reasonably clear. Various circumstances can mimic the inflammatory changes associated with Tb. lesions, and some of the most important are reviewed below.

Use of agents such as Xylazine has multiple effects on the circulating blood picture, and some of these have been reasonably well documented (e.g. Seal et al. 1972). In a series of controlled experiments we have observed a mean haemoglobin fall in adult hinds of 60 g/L, MCV changes, platelet count changes and a fall in lymphocytes of around 60% - an interesting feature of this latter result was a change in neutrophil/lymphocyte ratio in one animal from 0.7 to 2.2 within 45 minutes of intramuscular Xylazine administration.
Any known inflammatory condition, such as foot abscess, may confuse the picture, and should at least be noted on the return to the laboratory.

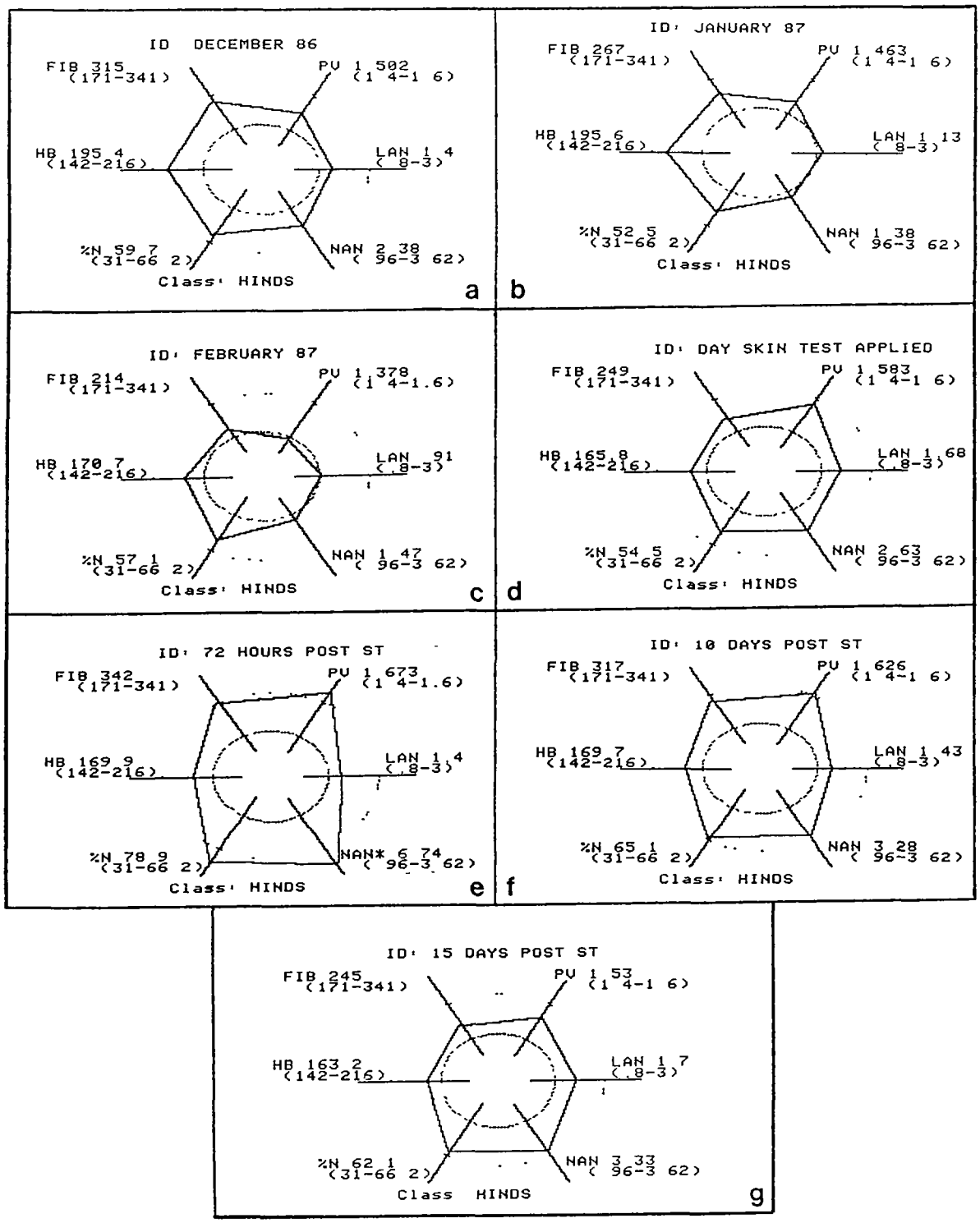

Fig 3 Effects of skin testing on haematological parameters in a deer from a suspect bovine herd

ABBREVIATIONS

FIB - fibrinogen
PV - plasma viscosity
LAN - Lymphocyte absolute number
NAN - Neutrophil absolute number
%N - percent neutrophils
HB - haemoglobin

Ideally, animals with such a condition should not be sampled until it has resolved.
It is well-known that physiological stress may raise the neutrophil count. This has been found in animals described as 'mad' by the handlers at the time of sampling. When investigating another case showing inflammatory signs for no clear reason, it was disclosed that the hind had twisted itself in the race shortly before sample collection. Clearly, if the first specimen happened to be taken at the time of reading the skin test, and the second at the time of some occult inflammatory episode, a false picture of lesion status might be presented.
For the purposes of the current experimental investigation, EDTA samples taken at the time of applying the skin test, and again when the skin test is read would be of considerable interest. Additionally, if the animal is to be sacrificed, a further sample taken a few days before the planned post-mortem to check the inflammatory status would be helpful.

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