FURTHER OBSERVATIONS ON XYLAZINE AND HAEMATOLOGICAL PARAMETERS IN RED DEER (*Cervus elaphus*): THE EFFECT ON REFERENCE VALUES, AND ON SPLENECTOMISED ANIMALS.

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INTRODUCTION.

Blood samples taken from healthy farmed red deer using xylazine sedation show a marked reduction in red cell mass, and there is a reduction in circulating lymphocytye numbers, platelet numbers, mean red cell volume, plasma viscosity and plasma fibrinogen (Cross, Mackintosh and Griffin, 1988). The fall in red cell mass has been described in other reports, mostly dealing with white-tailed deer (Chapman, 1977) or red deer not kept under farm conditions (Drescher-Kaden and Hoppe, 1972), and it has been suggested that the probable cause is that the spleen is contracted during physical restraint (Wilson and Pauli, 1982). This paper is a preliminary report on data, to be published in full elsewhere, describing results on sedated splenectomised deer compared to results on the same animals sedated prior to splenectomy. We also show basophils are affected by xylazine administration and present some reference values before and after sedation.

METHODS

Haematology - Haemoglobin, red cell count, leucocyte count, leucocyte differential and platelet count estimations, plasma viscosity and fibrinogen were performed as previously described (Cross, Mackintosh and Griffin, 1988; Cross, 1987). Basophil numbers on 10 adult hinds were also found using the Technicon H6000/C machine, and confirmed using manual differential counts (200 cells) performed on 10 other animals, before and after xylazine. Haptoglobin concentrations were found using the tetra-guaiacol method of Owen et al. (1960), modified for microtitre plates (Jones and Mould (1984) and using a deer haemoglobin preparation.

Reference ranges - Haematological reference ranges were based on the analysis of blood samples from apparently normal farmed red deer which were physically restrained and were selected for inclusion according to set 1 and set 2 variables as suggested by Elveback (1973).

Set 1 variables Sex. Age group. Presumptive *Cervus elaphus* - by external characteristics.

Set 2 variables No recent history of ill-health. Negative cell-mediated immunity tests for Avian and Bovine tuberculosis. Absence of intercurrent parasitic infestation as indicated by eosinophil count not greater than $0.3 \times 10^9/L$.

The nature of the distribution of each set of data was tested using the a_3 skewness statistic and Gearys 'G' statistic for kurtosis (Remington and Schork, 1970), and Lilliefors modification of the Kolmogorov-Smirnov test (Conover, 1971). Logarithmic transformation was used where necessary to convert the data to a Gaussian form. This was successful in all cases. Reference ranges were then calculated as 2 standard deviations on either side of the mean. Radial plot diagrams were prepared using an Apple Mackintosh computer, developed from an Apple IIe computer programme adapted by one of the authors (JPC) from that described by Love (1984).

Physical restraint - unsedated deer were either held manually or in a pneumatic

crush for blood collection.
Xylazine hydrochloride (Rompun, Bayer) - 1 mg/kg. was administered by intramuscular injection, and blood samples were collected prior to and 30 minutes after xylazine injection.
Splenectomy - Three 20 month old stags were splenectomised under Rompun/Fentaz sedation (Cross et al. in prep.). After removal the spleen was weighed and 1 mg adrenalin was injected into the splenic artery, and the blood expelled from the splenic vein was analysed.

RESULTS
Reference ranges - The general approach to haematological reference values used here has been discussed previously, (Cross and Heyns, 1983; Cross, 1983). A total of 230 weaners farmed under normal conditions and 7-8 months of age were tested Of these, 123 met all set 1 and set 2 criteria, and this group was analysed to give the data set out in table 1.

PARAMETER	RANGE	UNITS
Haemoglobin	160-213	g/L
Neutrophils	0.79-4.21	$x 10^9$/L
Lymphocytes	1 34-3.61	$x 10^9$/L
Basophils	0.04-0.22	$x 10^9$/L
Fibrinogen	1.67-3.87	g/L
Plasma viscosity	1.34-1.58	cp
Haptoglobin	0.05-0.17	HBBC mg/dL

TABLE 1: 95% Reference ranges for 7-8 month farmed red deer (N = 123).

The adult hind reference limits were derived from 100 mixed age adult hinds farmed under normal conditions on 4 different farms, with 42 sampled during the months June to September, 46 March to May and 12 between October to February (table 2).

PARAMETER	RANGE	UNITS
Haemoglobin	142-216	g/L
Red cell count	8.0-12.8	$x 10^{12}$/L
Neutrophils	0.96-3.62	$x 10^9$/L
Lymphocytes	0.81-3.02	$x 10^9$/L
Basophils	0.06-0.29	$x 10^9$/L
Fibrinogen	1.73-3.59	g/L
Haptoglobin	0.05-0.17	HBBC mg/dL

TABLE 2: Reference values for mixed age adult farmed red hinds (N = 100).

Ten hinds were tested before and 30 minutes after xylazine (table 3).

PARAMETER	PRE DRUG RANGE	30 MINS RANGE	UNITS
Haemoglobin	190-233	117-174	g/L
Red cell count	9.7-14.7	5.9-11.3	x 10^{12}/L
Neutrophils	1.28-2.6	1.25-2.85	x 10^{9}/L
Lymphocytes	1.59-3 2	0.98-2.3	x 10^{9}/L
Basophils	0.04-0 23	0.01-0.16	x 10^{9}/L
Fibrinogen	206-336	177-300	mg/dL
Plasma viscosity	1.44-1.59	1.34-1.51	cp
Platelets	223-423	158-333	x 10^{9}/L

TABLE 3: Comparison of pre-xylazine and 30 minute post-xylazine 'ranges' for 10 mixed age adult hinds - not suitable for use as true reference ranges.

While these results are presented in the form of reference values, it is important to realise that these animals were not selected using the defining criteria, and that the adult hind reference limits are as shown in table 2, not table 3.
The values for male deer (table 4) refer to 9-18 month old male farmed animals is derived from 37 animals of which 8 were sampled between March and May, 17 between June and September and 12 between October and February.

PARAMETER	95% RANGE	UNITS
Haemoglobin	132-196	g/L
Red cell count	9.15-13.0	x 10^{12}/L
Neutrophils	0.78-3.37	x 10^{9}/L
Lymphocytes	1.14-4.3	x 10^{9}/L
Basophils	0.08-0.35	x 10^{9}/L
Fibrinogen	172-388	mg/dL
Plasma viscosity	1.38-1.61	cp
Platelets	194-587	x 10^{9}/L

TABLE 4: Reference ranges for 37 male deer aged 9-18 months.

Xylazine in intact and splenectomised deer - A summary of the results found in intact and in splenectomised red deer is shown in fig. 1. Fig. 2 is a radial plot diagram indicating examples of the results obtained with pre- and post-xylazine blood samples plotted using pre-xylazine reference data.
Basophil counts - All 10 hinds tested using the H6000/C, showed a reduction in basophil numbers following xylazine administration, the mean pre-xylazine level being 0.14 x 10^{9}/L, while the post-xylazine mean was 0.04 x 10^{9}/L. A number of young male deer have been tested in this way (full results to be reported elsewhere). In six of these the fall was 0.2 x 10^{9}/L or more, the most extreme fall

being 0.34 x 10^9/L. The basophil changes indicated by studies using the Technicon H6000/C were confirmed by the results using manual differential counts, but the degree of error is high when manual techniques are employed, especially where cell numbers are low, so that the fall is less obvious Nevertheless, the manual technique also gave significant evidence of a basophil reduction ($p < 0.02$).

CONCLUSIONS

The value of laboratory test results is clearly enhanced by using precise reference values, though preparing these correctly is an expensive and time-consuming operation requiring expertise in a number of areas. The correct interpretation of results of such tests demands a detailed knowledge of the results for healthy animals of similar age and sex in the same season. It is also important to appreciate that the reference data points depend to a certain extent on the technology used, and can only be applied across technique differences with caution.

The reference ranges given here are for physically restrained deer, and marked difference can occur if the blood is taken from a sedated animal. This is highlighted in Fig. 2, which illustrates that several parameters may appear to be abnormal if blood from a sedated animal is compared to reference values from physically restrained deer.

In addition to being accompanied by a fall in red cell mass, platelets, fibrinogen and plasma viscosity, xylazine administration is associated with a very rapid and often precipitous fall in circulating basophil numbers as measured using the Technicon H6000/C haematology analyser and by manual differential counting Owing to the substantial degree of fall found in some animals, this effect could be a factor in stag deaths associated with xylazine sedation and velvet antler removal (Mackintosh and Cross, 1989).

Contrary to expectations, the fall in lymphocytes seen with xylazine is not due to the lymphocytes homing back to the spleen as it relaxes due to the sedation, since the effect of the drug on splenectomised animals is not significantly different to intact deer in this respect.

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