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CHEMICAL STUDIES ON BLOOD AND TISSUES FROM ANGORA GOATS CARRYING TO NORMAL TERM AND FROM HABITUALLY-ABORTING ANIMALS

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During the winter of 1958 the Onderstepoort Veterinary Research Institute was asked to conduct extensive field investigations into the problem of widespread abortions amongst Angora goats in the Cape Midlands. The condition had by then assumed alarming proportions. For this work use was made of a well-equipped mobile laboratory (Brown, 1959a). The team responsible for the investigations was thus able to conduct a comprehensive study of the bacteriology and chemical pathology of this condition. The bacteriological work is reported elsewhere (Van Heerden, 1961c); this paper records the results of the chemical studies.

Materials and Methods

A total of seventeen farms was visited during this investigation. On some of these farms habitual abortion apparently was quite unknown. The animals from these flocks were considered as normal controls for this work. Healthy, full-grown ewes which were either non-pregnant, on the point of kidding or had just kidded were selected. A number of these were bled for analytical purposes and then slaughtered in order to obtain material for chemical and histopathological purposes. Blood was collected using heparin or potassium oxalate as the anti-coagulants, depending upon the requirements of the particular determination to be made.

The majority of farms visited were those on which the condition was known to have been present for a number of years and on which the losses experienced were most serious. Goats were selected for study on the same basis as for the control group and were bled and slaughtered for the collection of material. In all instances where the animals were slaughtered a complete autopsy was conducted and the findings recorded. Freshly aborted foetuses, foetuses from habitually-aborting ewes and those from ewes with no history of previous abortion were collected for study.

All haematological procedures were carried out by standard methods and all colorimetric analyses were made using an EEL portable Model A photo-electric colorimeter. The following determinations were made on the blood samples collected, the methods being indicated in parenthesis: blood urea nitrogen (Brown, 1957), creatinine (Folin & Wu, 1919), uric acid (Carraway, 1955), blood amino acids (Hawk, Oser & Summerson, 1961), plasma bicarbonate (Van Slyke, Stillman & Cullen, 1919), plasma proteins (Weichselbaum, 1946; Kingsley, 1940), blood sugar (Lehman & Silk, 1952), plasma total calcium (Ferro & Ham, 1957), colloidal gold flocculation test (Gray, 1940; Maclagan, 1946), zinc sulphate turbidity test (Kunkel, 1947) and

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plasma bilirubin (Malloy & Evelyn, 1937). The following methods were taken from the book by King & Wootton (1956): haemoglobin, plasma inorganic phosphate, sodium, potassium, iron, cholesterol, the thymol turbidity and colloidal gold flocculation tests. Plasma copper was determined according to the method of Cartwright, Jones & Wintrobe (1945). Liver iron values were done using an unpublished method of the authors based on the procedures of Elvehjem (1930) and Kennedy (1927). Selenium analyses of foetal liver tissues were made using the method of Brown (unpublished 1962) which is based on those of Gortner & Lewis (1939) and Davidson (1939).

RESULTS

"Normal Control" Animals

Eighteen fully grown ewes from four different farms on which the condition was not known to have occurred previously, were used for this purpose. On all these farms management was of a high order and the goats were in good condition. Random fresh faeces samples taken from each flock and from the animals concerned in this work revealed only a moderate parasitic infestation. In most cases the endoparasites were identified as *Haemonchus, Trichostrongylus* and *Nematodirus* species. In a few instances eggs of *Ostertagia* species also were found. These findings were confirmed on autopsy of animals sacrificed for collection of material. In addition a moderate *Stilesia hepatica* infestation was found in a few animals.

The figures obtained from the six animals detailed in Table 1 are selected as representative of all the blood analyses carried out.

TABLE 1.—*Blood analysis from normal goats used as controls*

Determination	Goat No. 10	Goat No. 11	Goat No. 19	Goat No. 29	Goat No. 30	Goat No. 42
Red cell count (10^6/cu mm)	10·3	9·8	12·09	16·33	15·33	16·21
Red cell volume (%)	27	27	26	26	27	31
Haemoglobin (gm %)	8·4	8·8	9·1	9·5	8·8	9·8
Erythrocyte Sedimentation Rate (mm/hr)	1·0	1·0	2·0	2·0	2·0	1·0
Total leukocyte count (10^3/cu mm)	12·1	8·5	16·5	10·5	11·0	13·0
Absolute Eosinophile count (per cu mm)	220	120	112	340	260	500
Total Plasma Proteins (gm %)	5·20	5·33	5·76	5·87	5·55	4·59
Whole Blood Urea Nitrogen (mg %)	25·80	24·50	28·90	29·07	21·71	26·30
Whole Blood Creatinine (mg %)	0·35	0·10	0·75	0·20	0·50	0·50
Whole Blood Uric Acid (mg %)	1·00	1·73	1·23	4·50	1·73	2·48
Whole Blood Free Amino Acids (mg %)	8·50	6·17	8·00	6·52	8·92	5·33
Blood Sugar (mg %)	50·00	36·50	44·00	32·5	35·0	38·5
Total Plasma Cholesterol (mg %)	188·0	207·0	218·9	241·5	211·3	204.0
Plasma Sodium (meq/l)	144	144	144	144	144	147
Plasma Potassium (meq/l)	6·5	5·6	6·7	4·5	4·6	4·6
Total Plasma Calcium (mg %)	9·00	10·5	12·11	11·58	9·47	10·50
Plasma Magnesium (mg %)	2·99	3·95	2·66	2·50	2·10	2·22
Plasma Iron (mcg %)	150·0	150·0	222·0	109·1	118·2	127·3
Plasma Copper (mcg %)	70·0	70·0	88·0	100·0	75·0	75·0
Plasma Chloride (meq/l)	97·0	105·0	98·2	104·3	106·4	100·00
Plasma Bicarbonate (meq/l)	25·0	25·9	24·1	24·0	24·0	24·0
Plasma Inorganic Phosphate (mg %)	4·30	4·79	4·36	5·09	3·85	2·91
Total Plasma Bilirubin (mg %)	0	0	0	0	0	0
Thymol Turbidity (Maclagan Units)	0·8	1·0	0·8	1·0	0·8	0·8
Zinc Sulphate Turbidity (Kunkel Units)	1·5	2·9	1·5	2·2	1·5	0·8
Colloidal Gold Flocculation	0	0	0	0	0	0

Evidence of an anaemia which varied from mild to moderate was apparent in most of the goats. This in all probability was attributable to the parasitic infestation. In the majority of animals the plasma copper values were slightly below the accepted normal limits for goats, viz. 80–120 mcg per cent for animals in the Cape Midlands. In a few instances (e.g. No. 29) evidence of mild kidney damage, as revealed by slight elevations of urea nitrogen and uric acid, was encountered. A few other ewes, not shown in Table 1, showed some signs of chronic liver disease as indicated by positive colloidal gold flocculation tests and zinc sulphate turbidity readings of over 7 Kunkel units. These tests have been found most useful by the authors for the diagnosis of chronic hepatic dysfunction in sheep and goats (unpublished observations). Both the kidney and liver disturbances were confirmed at autopsy and will be discussed later.

With the exception of the figures pertaining to the erythrocytes and the plasma copper levels, the values cited in Table 1 may be taken as representative of normal values for full-grown Angora ewes in the Cape Midlands. No difference in the various figures obtained was evident between goats at various stages of pregnancy and non-pregnant goats.

Habitually-aborting ewes and other pregnant ewes from farms on which the condition is most prevalent

Animals from fourteen severely affected farms were used for this part of the work. Non-pregnant females, ewes which had either just kidded normally or aborted and goats near to termination of gestation were bled. No significant difference between the three groups was found for any of the figures obtained and the results are represented by those from twelve ewes shown in Table 2.

Random faeces samples from the flocks out of which these goats were taken and from all the animals examined, revealed more or less severe intestinal parasitisation by *Ostertagia, Trichostrongylus, Nematodirus* and *Oesophagostomum* species. These finds were confirmed on autopsy. With a few notable exceptions the farms visited were overstocked, the grazing in some instances had deteriorated alarmingly and the general level of flock management was not as good as on the farms from which the control animals were taken.

Consideration of the results presented in Table 2 again shows anaemic changes associated presumably with the internal parasitism. Evidence of early renal lesions was shown by some goats as indicated by the mild to moderate elevations of urea nitrogen, and/or uric acid depending upon the stage of the condition, e.g. No. 8, 13, 15, 22 and 23. In some of these animals, notably No. 2, 13, 14 and 32, signs of chronic liver damage, as revealed by positive colloidal gold flocculation tests were seen. In all instances the co-existent or separate renal and hepatic lesions were confirmed on autopsy. Kidney lesions were generally nephrotic in nature. In one instance (not shown in the table) a subacute nephritis with cystic degenerative changes was evident. The liver damage encountered varied from mild to severe degenerative lesions or advanced cirrhotic changes.

Many of the goats shown in Table 2 and other animals of this group appear to exhibit some degree of plasma electrolyte imbalance. In some cases the abnormalities seen may possibly be attributed to the renal lesions present, e.g. No. 22 or 23, or to the existence of severe liver lesions, e.g. No. 14 and 32. Little significance was attached, however, to the variations in the electrolyte figures, since these may have reflected current stresses to which the animals were subject, e.g. environmental conditions, impending parturition or abortion, etc.

TABLE 2.—*Blood analysis from Angora ewes from farms on which abortion occurs to a more or less severe degree*

Determination	Goats Numbers											
	6 Non-pregnant	8 Non-pregnant	15 Non-pregnant	23 Non-pregnant	2 Pregnant	5 Pregnant Habitual Aborter	32 Pregnant	51 Pregnant (twins)	13 Aborted	14 Aborted	22 Pregnant Dead foetus	40 Aborted
Red Cell Count (10^6/cu mm)	11·7	12·24	8·17	15·49	8·43	9·1	10·90	15·82	17·29	8·60	20·11	12·36
Red Cell Volume (%).....	33	29	34	28	34	27	23	33	32	25	29	37
Haemoglobin (gm %).....	9·1	9·8	11·0	9·5	10·1	8·8	9·8	11·3	11·1	7·5	9·8	11·2
Erythrocyte Sedimentation Rate (mm/hr).....	1·0	1·0	1·0	0	1·0	1·0	2·0	1·0	1·0	1·0	0	1·0
Total Leukocyte Count (10^3/cu mm).....	10·90	10·85	6·3	15·25	12·25	7·05	14·85	17·6	6·85	13·05	18·08	8·55
Absolute Eosinophile Count (per cu mm).....	102	460	680	880	200	420	20	20	240	540	40	140
Total Plasma Proteins (gm %)	5·97	5·76	5·33	5·87	5·55	8·76	5·65	5·33	6·29	6·08	5·65	4·79
Whole Blood Urea Nitrogen (mg %).....	18·03	14·72	21·34	37·16	18·76	14·72	17·80	27·20	18·95	24·47	31·64	23·70
Whole Blood Creatinine (mg %).....	0·12	0·40	0·40	0·60	0·50	0·10	0·30	0·25	0·25	0·10	0·90	1·50
Whole Blood Uric Acid (mg %).....	2·48	5·24	7·76	4·00	1·73	0·62	0·50	2·48	3·50	6·26	6·48	0·50
Whole Blood Free Amino Acids (mg %).....	5·67	5·67	4·40	7·20	8·34	9·00	5·49	4·67	5·05	3·20	4·93	6·27
Blood Sugar (mg %).....	33·00	33·00	43·00	59·50	54·00	53·50	85·50	87·50	63·50	65·5	148·0	115·0
Total Plasma Cholesterol (mg %).....	207·5	184·9	188·70	234	226·4	203·70	241·5	272·2	177·3	207·5	207·5	172·2
Plasma Sodium (meq/l).....	132	147	144	141	138	144	144	150	153	138	141	156
Plasma Potassium (meq/l)..	5·1	5·9	4·1	4·6	5·8	6·0	4·5	4·4	5·9	4·3	5·6	4·9
Total Plasma Calcium (mg %)	10·00	9·47	10·00	9·47	10·52	10·00	11·58	10·52	8·42	9·47	9·47	10·52
Plasma magnesium (mg %)	2·34	2·42	2·30	2·58	5·32	2·50	2·99	2·42	2·99	2·38	2·42	2·22
Plasma Iron (mcg %).....	222	200	200	63·64	175	200	63·64	81·82	125	200	72·74	222
Plasma Copper (mcg %)...	82	100	100	125	75	80	88	60	88	80	75	75
Plasma Chloride (meq/l).....	126·00	120·00	96·40	97·31	92·80	92·00	111·60	105·40	96·40	95·50	97·30	107·3
Plasma Bicarbonate (meq/l)..	18·75	23·21	20·98	18·00	24·01	25·89	21·00	20·98	24·10	27·00	18·00	26·00
Plasma Inorganic Phosphate (mg %).....	3·56	3·93	4·00	4·29	4·79	3·78	1·30	2·40	4·29	2·40	4·44	2·91
Total Plasma Bilirubin (mg %).....	0	0	0	0	0	0	0	0	0	0	0	0
Thymol Turbidity (Maclagan Units).....	0·8	0·8	1·0	1·0	0·8	0·8	1·0	0·8	0·8	0·8	0·8	0·8
Zinc Sulphate Turbidity (Kunkel Units).....	2·2	2·2	1·5	2·9	4·3	2·9	3·6	1·5	1·5	2·2	2·9	2·2
Colloidal Gold Flocculation	0	0	0	0	+	0	+++	0	+	+	0	0

Strikingly low plasma inorganic phosphate values were found in many of these ewes, as illustrated by goats No. 14, 32, 40 and 51. Plasma copper values throughout the group were in general either of the order of the accepted lower normal limit for this element or slightly below this figure. Some of the animals represented by ewes No. 22, 23 and 32, exhibited very low plasma iron levels. This may have been a reflection of the intestinal parasitism which was severe in these animals, coupled with the normal physiological drain of maternal iron to the foetus in those which were pregnant. A probable example is ewe No. 51 which carried twins.

In some animals fairly high blood sugar values were noticed, e.g. No. 22, 32, 40 and 51, the figures for No. 22 and 40 representing frank hyperglycaemia. Both animals showed glycosuria. No particular significance was attached to these findings, since they were probably purely incidental.

Iron determinations on foetal livers

Since low plasma iron values were encountered in many goats from farms on which abortions were prevalent, it was decided to compare the liver iron content of pathological foetuses with that of normal foetuses of the same age. Tissues from freshly aborted or dead foetuses, taken from ewes at autopsy, were compared with those obtained from healthy foetuses of the control animals. The results are presented in Table 3.

TABLE 3.—*Foetal liver iron values*
(All figures presented are in terms of mg per 100 gm of wet liver tissue)

Nature of Foetus	No. of Foetus	Age of Foetus	Liver Iron
Normal healthy living foetuses.....	A	3 months	37·78
	B	3 months	30·22
	C	3½ months	26·68
	D	2½ months	37·78
	E	1½ months	13·78
Foetuses either aborted or found dead *in utero*.....	F	3½ months	11·11
	G	3½ months	16·00
	H	3 months	7·11
	I	2½ months	3·56
	J	2½ months	11·55
	K	2½ months	6·98
Normal living twin foetuses.....	L1	2½ months	14·22
	L2	2½ months	8·89

It is apparent from the figures shown that the values obtained from aborted foetuses or foetuses found dead *in utero* at autopsy are far below those of healthy viable foetuses of the same age. It is of interest to note that the twin foetuses, designated L1 and L2, were taken from ewe No. 51 in which the plasma iron level was found to be 81·8 mcg per cent. In all other instances maternal iron levels lay between 100 and 250 mcg per cent. In this particular case severe intestinal parasitism and thus a severe drain on the mother's iron reserves, undoubtedly contributed towards the low foetal liver iron values found.

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Selenium detemination on foetal livers

During the course of a separate subsequent investigation Brown & de Wet (1962) examined some of the remaining foetal liver material for the presence of selenium. Some of the results of these analyses are shown in Table 4.

Although the number of foetuses examined was small and the results should thus be interpreted with caution, it would appear that the same relationship holds as in the case of iron above, i.e. the pathological foetuses have apparently lower liver selenium values than normal foetuses of the same age from the same area.

Discussion

For most of the blood constituents determined, little difference was found between the control animals and those from farms where abortion was prevalent to a more or less severe degree.

Van Heerden (1961a, b) in his studies on the problem has advanced the theory of an inherent weakness of the hypophyseal-gonadal axis. This was characterised by premature regression of the corpus luteum verum consequent to inadequate secretion of luteotrophic hormone. Evidence supporting this hypothesis has been obtained by Brown, van Rensburg and Gray (1963) during a study of the urinary excretion of 5β-pregnane-3α:20α-diol in pregnant Angora goats.

Table 4.—*Selenium analyses on Angora foetus liver tissues*

(All results are expressed as micrograms (mcg) selenium per gm of tissue on a wet basis. All foetuses were of approximately the same age, viz. 2 to 3 months).

Foetus No.	Nature of Foetus	Liver mcg Se
3	Normal foetus	31·0
36	Normal foetus	14·0
38	Normal foetus	17·1
35	Aborted foetus	6·0
40	Aborted foetus	0·0
43	Aborted foetus	5·5

Although the work reported in this paper sheds little light upon the actual causes of abortion in goats, it is of value in indicating some of the secondary factors which may contribute towards the high incidence of the condition particularly on farms where management and level of nutrition leave much to be desired. Many of the points made by van Heerden are confirmed.

Verminosis and probably coccidiosis occur to a greater or lesser degree amongst goat flocks throughout the affected areas, the animals examined all showing varying degrees of anaemia. Although there is no direct correlation between this finding and the incidence of abortion, it is evident that maternal sideropaenia is a factor which may be of considerable importance in neonatal mortality amongst kids in these areas.

The striking difference between the liver iron content of normal and pathological foetuses is significant. In the light of the finding of van Heerden (1961a, b) and Brown *et al.* (1963), these figures may be interpreted as evidence of some impairment of placental transfer of nutrients to the foetus arising early in gestation.

Although the mothers of all the foetuses examined exhibited varying but mild degrees of anaemia, they were not, with the exception of No. 15, sideropaenic and it may be assumed that in most instances the maternal iron reserves should have been sufficient to satisfy the demands of the growing foetus.

The same argument may be applied equally to an interpretation of the results of the selenium analyses presented in Table 4. It is also unlikely from inspection of these results and from other considerations that excess selenium in the vegetation of these areas (Brown & de Wet, 1962) is in any way concerned with the aetiology of the condition other than in a purely secondary role.

The very low plasma inorganic phosphate values seen in some goats must be interpreted with caution. There is no direct evidence emerging from this investigation to indicate that a phosphorus deficiency is present in any of the affected flocks or in the vegetation of this area. Factors interfering with the intestinal absorption of this element, e.g. chronic enteritis consequent to verminosis or coccidiosis, excessive calcium intake from the water of some farms and other dietary factors such as the injudicious use of trace element mixtures, etc. may all contribute towards the hypophosphataemic states seen.

On the other hand the almost universally low plasma copper levels encountered may suggest that in the areas investigated suboptimal copper intake may be an important contributing factor.

The liver and kidney lesions mentioned have been encountered in apparently normal sheep and goats throughout most of the Karoo and Cape Midlands and have been described in earlier papers by Brown and co-workers (Brown & de Wet, 1962; Brown, le Roux & Tustin, 1960; and Brown, 1959b). The incidence of these lesions bears no relationship to the occurrence of verminosis or other intercurrent infections but appears to be entirely due to dietary factors. Excess selenium in the Karoo vegetation has been mentioned as a possible hazard in this respect (Brown & de Wet, 1962).

Although chronic hepatic disease appears to be fairly ubiquitous in the Karoo and Cape Midlands, its possible role in the aetiology of Angora goat abortions cannot be dismissed lightly. It must be remembered that the liver is an important site for the detoxication of steroid hormones. It is possible that the co-existence of chronic liver damage and the hereditary hormonal imbalances postulated by van Heerden may serve to increase the incidence of abortions in these animals. Failure to detoxicate any oestrogens formed during the latter two-thirds of gestation, especially if progesterone secretion is suboptimal during the first third of term (Brown *et al.*, 1963) may have serious consequences regarding the outcome of gestation.

No evidence was obtained from this work that any infectious agent is responsible for the condition, lending thus further weight to van Heerden's conclusions.

SUMMARY

The results of a comparative study of the haematology and the levels of various constituents of blood taken from certain groups of ewes emanating from farms where abortion was either not known to have occurred previously and where it was most prevalent, are reported. Anaemia consequent to verminosis, sideropaenia and chronic hepatic disease are cited as possible contributing factors in the aetiology of Angora goat abortions and neonatal kid mortality. A marked difference in the

liver iron and selenium content between normal and pathological foetuses has been observed. This is interpreted as evidence of early impairment in the placental transfer of nutrients between mother and foetus. Hypocupraemia was encountered in most of the animals studied. Copper deficiency in the areas concerned may be an important secondary factor on some farms.

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