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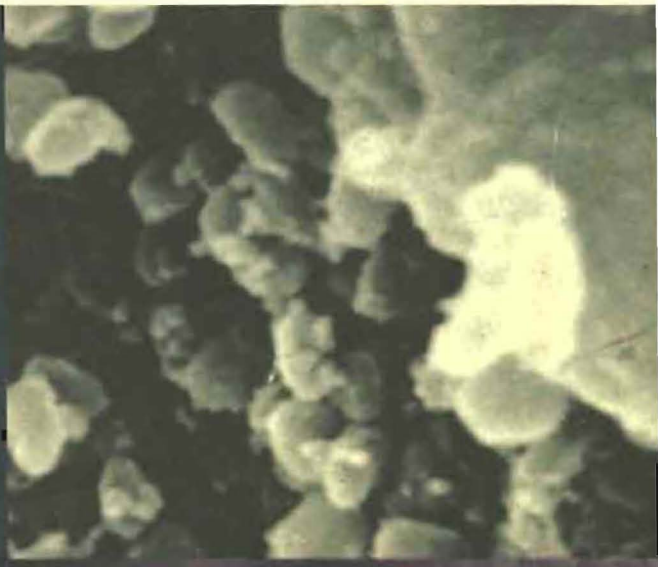

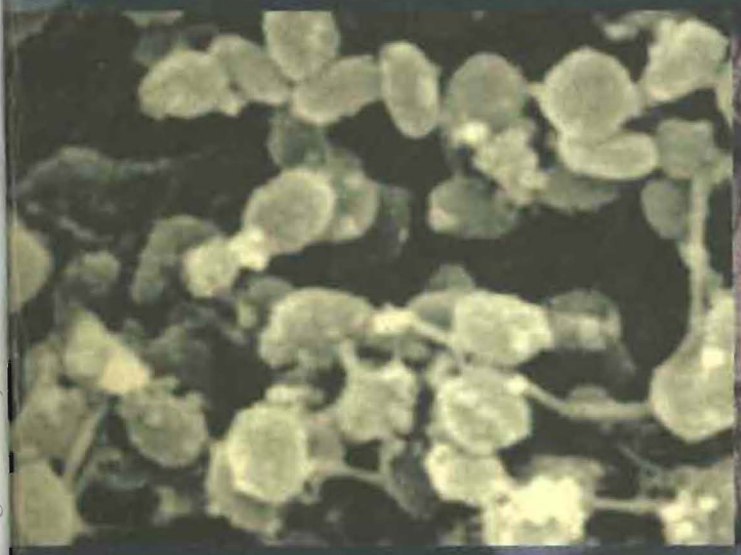

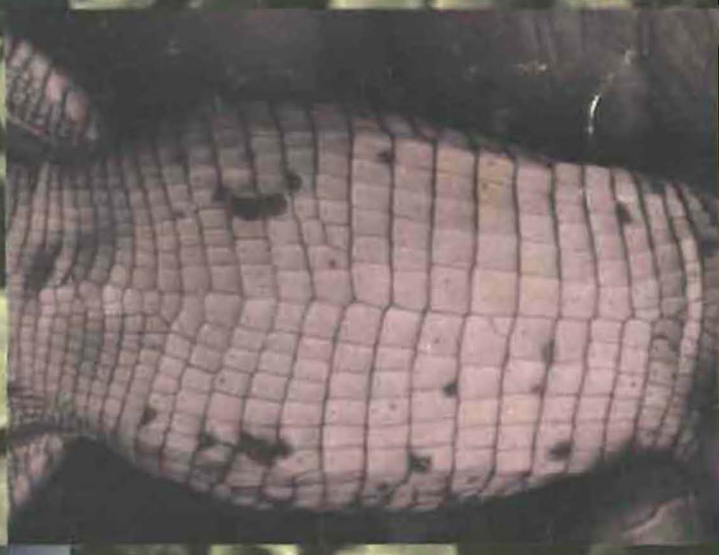

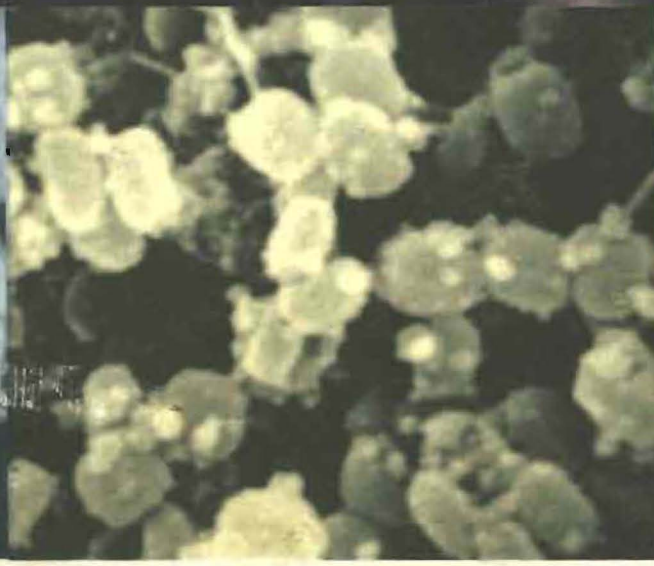

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INVOLUTION OF THE POST PARTUM UTERUS OF THE BOER GOAT

J P C GREYLING* and C H VAN NIEKERK**

ABSTRACT

The microscopic uterine involutionary changes were studied in Boer goats (n = 16) from 2 h to 34 d post partum. The endometrial epithelium showed a linear decrease in thickness with time post partum. A highly significant negative correlation (r = -0,94; P < 0,01) was found between the endometrial epithelium layer thickness and time post partum. The lamina propria layer showed a rapid decrease during the first 20 d post partum and then tended to level out. According to the statistical model ($y = Ae^{Bt+Ct^2}$) fitted by 25,2 d post partum, the thickness of the lamina propria layer was static.. The myometrium decreased significantly (P < 0,01) in thickness from Day 24 to Day 34 post partum. The thickness of the serosa followed a linear-type decrease during the observation period, while in the glandular epithelium the decrease was rapid during the first 12 d following parturition. The uterine glands reached normal size 22,1 d after parturition. The involution process of the uterus was microscopically complete by Day 28 post partum, the main indicator being the degree of recovery of the endometrial epithelium over the caruncular areas.

Key words: Boer goat, uterine involution, post partum, histology.

Greyling J.P.C.; Van Niekerk C.H. **Involution of the post partum uterus of the Boer goat.** *Journal of the South African Veterinary Association* (1991) 62 No. 1, 4-9 (En.) Department of Animal Science Faculty of Agriculture, University of the Orange Free State, P.O. Box 339, 9300 Bloemfontein, Republic of South Africa.

INTRODUCTION

The productivity of any breeding female is determined by the number of progeny delivered in a given period of time. The interval from parturition to a subsequent pregnancy is a factor of major economic importance and hence the involution of the post partum uterus must be seen as one of the important limitations in achieving the goal of optimal reproductive efficiency.

Histologically, the uterine wall consists of 3 layers, namely the endometrium, myometrium and the perimetrium. The endometrium consists of a layer of simple columnar epithelium, with a lamina propria layer under the epithelial layer. The mucous membrane lining the uterine body and uterine horns have numerous prominent small round cupshaped caruncles embedded in the surface. The whole lamina propria is richly supplied with blood vessels, with the larger vessels being located towards the myometrium. Tubular glands are present throughout the lamina propria, except in the caruncular areas in ruminants. The myometrium consists of a thick inner circular and an outer longitudinal layer of smooth muscle cells, which increase in number and size during pregnancy. Deep in the inner layer, is a vascular zone consisting of large arteries, veins and lymph vessels. These vessels communicate with the endometrium. The serosa is composed of loose connective tissue covered by the peritoneal mesothelium. Smooth muscle cells and numerous lymph ducts, blood vessels and nerve fibres are present in this layer[1 5 13].

After distension and distortion of the uterine tissues during pregnancy and the heightened glandular development required to support the conceptus, the uterus must undergo contraction and loss of weight, together with extensive regeneration of its epithelial layers during the process of uterine involution[9]. The drastic drop in the mass and volume of the uterus of the ewe during the first 8 d following parturition, can be ascribed to the contraction of the myometrium, vasoconstriction and the loss of tissue fluids[1 6].

In contrast to sheep[2 12 13], relatively little has been reported on involution of the post partum uterus of the goat. According to Van Wyk[13] and Botha[2], uterine involution in sheep is complete 28 d to 34 d post partum. Most studies have however focused on the endocrinological status of the goat before and around partus[3 4 11].

This study therefore aims to describe histological changes in the uterus of the Boer goat after parturition.

MATERIALS AND METHODS

Multiparous Boer goats (n = 16) were slaughtered, one by one, at the following times after parturition: 2, 12, 24, 36 and 48 h, as well as 4, 8, 12, 16, 20, 24, 26, 28, 30, 32 and 34 d. The body mass of all animals was recorded prior to slaughter. The reproductive tract was removed and 2 tissue samples of the caruncles, with a section of the inter-caruncular area (at the bifurcation), were subsequently taken from each uterine horn and fixed in 10% buffered formalin solution. Following fixation and embedding, section of 6 to 10 μm were cut from all the specimens and they were stained according to the haematoxylin-eosin technique[8]. The sectional thicknesses of the different histological layers were microscopically measured with the aid of a calibrated eyepiece. Each layer was measured in 10 different positions on a line perpendicular to the layers being measured, and these measurements were converted and expressed in terms of μm. Each specimen was also histologically examined and the micro-anatomy described.

The microscopic involution process of the post partum uterus was described and predicted by the equation $y = Ae^{Bt+Ct^2}$, where y = parameter involved; A = value of parturition; B + C = constants; t = time interval post partum; e = base of the natural system of logarithms[10].

RESULTS

The body mass of goats is presented in Table 1 and the thickness of the different

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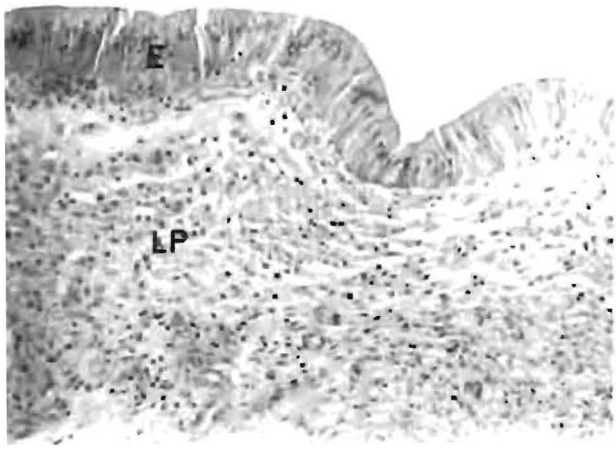

Fig. 1A: **The uterine endometrium 12 h post partum. The endometrium of the intercaruncular area is convoluted. Vacuoles are present in the cytoplasm of the epithelial cells. E - endometrial epithelium; L P - lamina propria (x 200)**

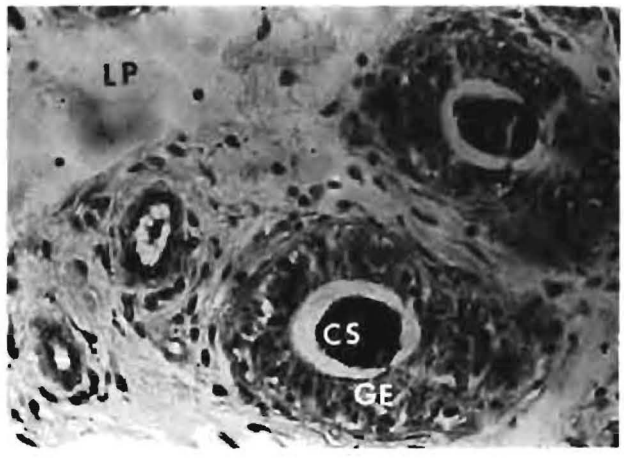

Fig. 1B: **The lamina propria 12 h post partum. The uterine glands are large and contain cell secretions, with vacuoles in the glandular epithelium. L P - lamina propria, G E - glandular epithelium; C S - cell secretion (x 320)**

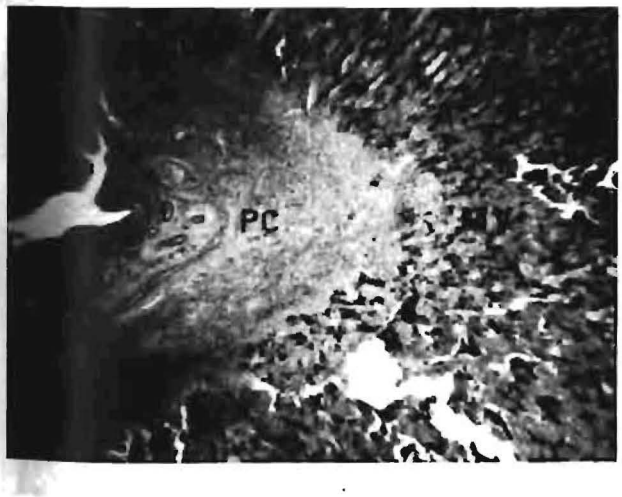

Fig. 1C: **The caruncular area 4 d post partum. The permanent caruncular tissue is contracted at the base of the maternal villi, which have degenerated. The proximal area of the maternal tissue is an amorphous mass of connective tissue. P C - permanent caruncular tissue, M V - maternal villi (x 12)**

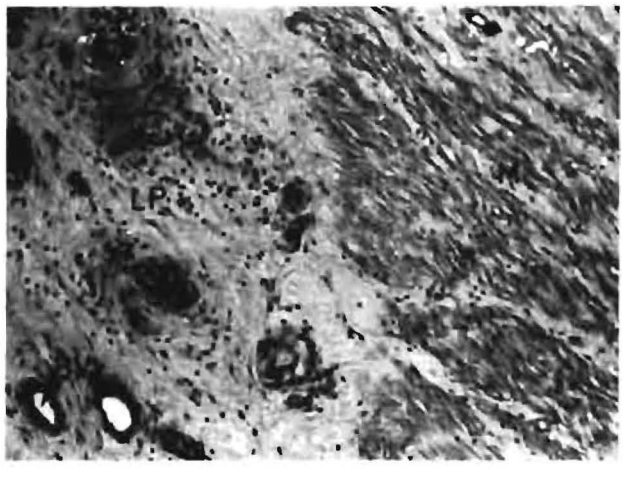

Fig. 1D: **The intercaruncular area 4 d post partum indicating the distinct border between the lamina propria and myometrium. L P -lamina propria; M - myometrium; B V -blood vessel (x 40)**

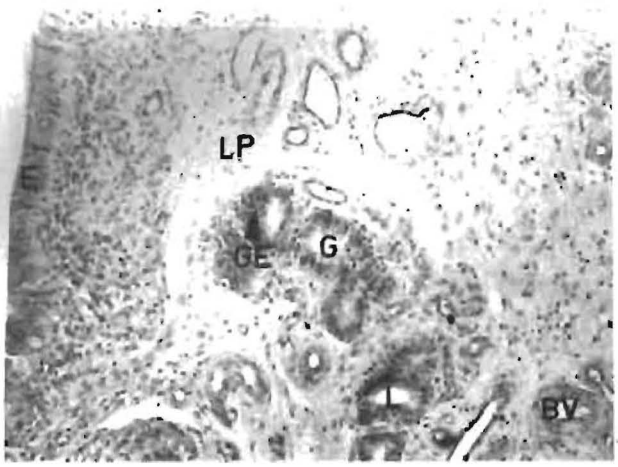

Fig. 2A: **The endometrium in the intercaruncular area 12 d post partum. The epithelium is convoluted and the lumen of the glands in the lamina propria contain cell secretions. E - endometrial epithelium; LP -lamina propria; G - uterine gland; BV -blood vessel; L - glandular lumen; GE -glandular epithelium (x 100)**

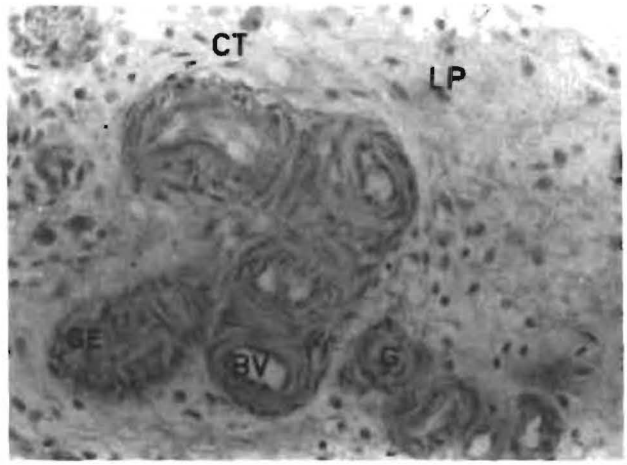

Fig. 2B: **The endometrial lamina propria 20 d post partum. The lamina propria consists of loose connective tissue with fibroblasts present and uterine glands with a few leucocytes present in the lumens of the glands. LP -lamina propria; G - uterine gland; GE - glandular epithelium; BV - blood vessel; CT - connective tissue (x 200)**

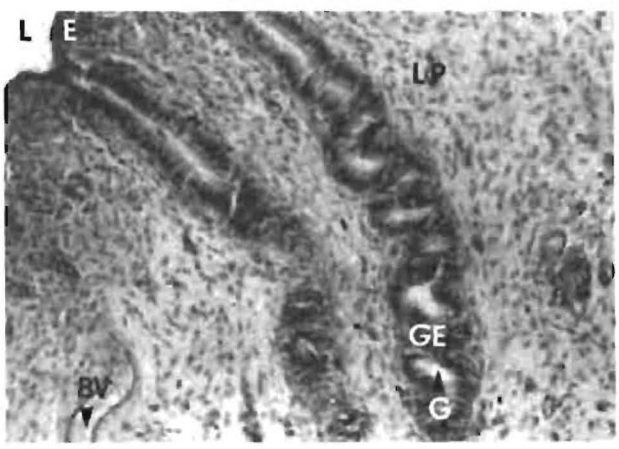

Fig. 2C: **The endometrium 24 d post partum. The coiled tubular glands are conspicuous. E -endometrial epithelium; LP - lamina propria; GE -glandular epithelium; BV -blood vessel; L - uterine lumen (x 100)**

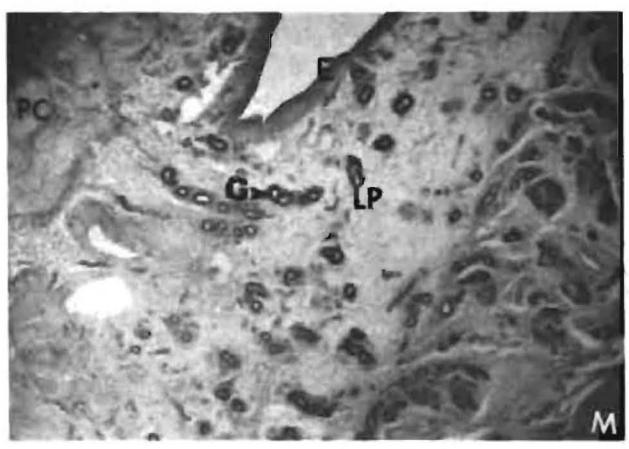

Fig. 2D: **The caruncle 34 d post partum. The endometrial epithelium is completely restored over the caruncle. E - endometrial epithelium; LP - lamina propria; G -uterine gland; PC - permanent caruncular tissue; M - myometrium (x 20)**

histological layers in Tables 1 & 2. The corresponding values attained by implementation of the equation $y = Ae^{Bt+Ct^2}$ for microscopic histological changes occurring in the post partum uterus, are presented in Table 3.

The endometrial epithelium (Table 1) of the uterine wall showed an almost linear decrease in thickness as time progressed, with a highly significant negative correlation existing between layer thickness and time post partum ($r = 0,94$; $P < 0,01$). Similarly, the thickness of the lamina propria showed a rapid decrease during the first 20 d post partum, and gradually decreased over the next 5 d to be complete after 25,2 d post partum (Table 1 and 3). The thickness of the glandular epithelium decreased rapidly during the first 12 d following parturition, whereafter the changes were much less marked (Table 2). A significant correlation ($r = 0,73$; $P < 0,01$) existed between the thickness of the glandular epithelium and the time post partum. The diameter of the uterine glands in the lamina propria showed a decrease of 6,3 ± 1,5% per day over the 34 d post partum observation period, with the normal original size being reached 22,1 d following parturition (Table 3). The myometrium on the other hand, decreased significantly ($P < 0,01$) in thickness from Day 24 (3 072,9 μm) to Day 34 (781,3 μm) post partum. The thickness of the serosa layer followed a linear-type decrease between parturition and 32 d post partum (Table 1).

The micro-morphological changes occurring in the caruncular and intercaruncular areas, from parturition to 34 d post partum, can be described as follows (The main indicator of the stage of the involutionary process was the degree of recovery of the endometrial epithelium over the caruncular areas): from parturition to 24 h post partum, the permanent caruncular tissue was horizontally spread out relative to the endometrium, with a hyalinised connective tissue layer showing constricted vascularisation, situated dorsally (lying ventral to the maternal villi). The columnar epithelium in the intercaruncular area was convoluted and the cytoplasm of the epithelial cells vacuolated. The lamina propria was richly vascularised and contained large uterine glands, filled with cell secretions and leucocytes (Fig. 1A, B). The thickness of the lamina propria was slightly less 12 h post partum than at parturition (Table 1). At parturition, the proximal area of the maternal villi were distinguishable as projections with cavities where the foetal villi were located, although no residual foetal villi could be identified as such. Cellular debris was present in some cavities. By 24 h post partum, the distal area of the maternal villi had degenerated into an amorphous mass, while in the proximal areas most of the hyalinised villi had retained their original structure. Epithelial cells of the maternal villi could still be distinguished, although many of their nuclei were pycnotic. By 36 h post partum, in the proximal area of the permanent caruncle tissue, the blood vessels were constricted as a result of hyalinisation in the artery walls. By 4 d post partum, the permanent caruncular tissue (Fig. 1 C) had contracted, with a hyalinised connective tissue layer situated dorsally being prominent. The epithelial layer of the endometrium in the intercaruncular area was convoluted, with vacuoles in the epithelium cells occurring in this post partum period. Where the epithelial cells of the proximal area of the maternal villi were still readily discernible 36 h post partum, the epithelium cells had almost completely degenerated by 4 d post partum, having an amorphous appearance. The thickness of the different histological layers (Fig. 1D) showed a steady decline from parturition to 4 d post partum. The lumens of the uterine glands in the lamina propria had also decreased in diameter during this stage (Table 2) and contained cell secretions and leucocytes. By 16 d post partum, there were indications that the epithelial endometrium of the intercaruncular area was starting to progess from both sides over the permanent caruncular area, just underneath the hyalinised band of connective tissue which was loosely attached to the permanent caruncular tissue (Fig. 2A). Fragments of necrotic tissue had broken loose from the permanent caruncular tissue and there was a definite border between the necrotic tissue and caruncular tissue. At this stage, the columnar epithelium of the endometrium had decreased in thickness and was less convoluted than at any previous stage. During the period 20 d -26 d post partum, the permanent caruncular tissue had contracted to its normal mass of fibroblast cells and fibres. The hyalinised band of connective tissue, together with the rest of the necrotic tissue derived from degeneration of the maternal villi, had come loose and were expelled through the cervix by Day 20 post partum. By Day 20 post partum, the diameter of the uterine glands and thickness of the glandular epithelium (46,3 μm and 18,7 μ respectively) were approximately those of the non-pregnant uterus (53,4 μm and 16,9 μm respectively)[7]. At 26 d post partum, some uterine glands still contained secretions in their lumens. Individual variation existed in the thickness of the endometrial epithelium, lamina propria, myometrium and serosa layers between the 20 d - 26 d post partum period (Table 1), but in all cases it was substantially less than at parturition. By 26 d post partum, the endometrial epithelium covered the entire caruncle surface, although the columnar epithelium did not appear to have the same thickness over the entire caruncular surface (Fig. 2B, C).

During the latter stages of the observation period (28 d - 34 d post partum), the cross-sectional thickness of the columnar epithelium and lamina propria of the endometrium was similar to that of the normal non-pregnant goat uterus (19,2 μm and 1192,7 μm respectively) (Table 1). Leucocytes present in the uterine glands of the lamina propria during this period were sparsely distributed. The number of glands per unit surface area (Table 2) in the lamina propria had increased (due to a decreased diameter in lumen of the glands) to a mean count of 60,6 per unit surface area. The endometrial epithelium was fully restored over the entire caruncular areas by Day 28 and by 34 d post partum, the myometrium and serosa layers were also completely normal (Fig. 2D).

DISCUSSION

By Day 28 post partum, the epithelium and the lamina propria of the endometrium in the intercaruncular area were of normal thickness and compared well to the normal non-pregnant goat uterus. Similarly, the uterine glands and glandular epithelium at this stage were similar to those of the non-pregnant goat[7] - suggesting that the endometrium was completely involuted at this stage. This ties in with the statistical model fitted, according to which the post partum changes in the thickness of the lamina propria layer are static 25,2 d post partum. The initial rapid decrease in thickness of the lamina propria can be attributed to a loss in tissue fluid, due to the connective tissue cells of the lamina propria becoming more tightly grouped, vasoconstriction and the involution of the uterine glands[2]. The glandular epithelium (Table 2) showed a relatively rapid recovery in terms of cell layer thickness - which is in agreement with the findings of Van Wyk[13]. The myometrium decreased significantly in thickness from Day 24 to Day 34 post partum, thus, relatively late post partum. By Day 28 post partum, the myometrium and serosa were slightly thicker than in the non-pregnant animal. The serosa followed a linear decrease between parturition and Day 32 post partum, before starting to level off at Day 34. The serosa, composed of loose connective tissue (although smooth muscle cells, lymph, blood vessel and nerve fibres are also present in this layer) could be partly responsible for its relatively slow recovery rate, due to the connective tissue taking longer to become more compact.

In the caruncular area, the permanent

Table 1: **The mean microscopic cross sectional thickness (μm) of the different histological layers in the uterus of the post partum Boer goat**

Goat No.	Goat mass (kg)	Birth status	Days post partum	Endometrial epithelium (μm)		Lamina propria (μm)		Myometrium (μm)		Serosa (μm)	
				LH	RH	LH	RH	LH	RH	LH	RH
R24	67,2	2	0,1	46,7	40,4	3 399,7	3 544,9	2 430,6	2 987,5	231,0	179,0
R23	62,5	1	0,5	46,7*	42,2	2 938,3*	2 923,4	2 203,1	2 118,8	274,5	232,1
R30	54,8	2	1	39,0	38,7	3 601,1	3 446,6	1 725,0	1 850,0	186,7	220,8
R3	58,7	1	1,5	36,8	37,8*	2 232,3	2 242,1*	2 187,5	1 571,4*	256,0	200,0*
R27	57,0	2	2	35,2	35,5	1 671,8	1 557,1	2 343,8	1 843,8	262,4	224,0
R13	65,8	2	4	34,9	32,0	1 389,9	1 376,8	2 968,8	2 357,3	180,6	146,7
R5	54,3	2	8	31,7	30,4	870,5	947,0	2 593,8	2 734,4	144,5	113,5
R9	55,5	2	12	34,9	35,2	1 262,0	1 491,5	1 562,6	2 431,4	105,1	126,0
R10	58,6	3	16	27,5	29,1	832,6	953,9	3 304,7	2 968,8	113,4	94,6
R28	50,4	2	20	22,7	25,9	1 396,4	1 022,7	2 156,3	2 687,5	94,2	78,6
R15	55,8	2	24	24,6	23,0	943,2	1 098,1	2 968,8	3 176,9	99,2	104,7
R17	51,4	2	26	24,6	25,9	845,7	953,9	1 109,4	1 238,4	48,8	47,2
R8	54,1	2	28	23,0	24,6	960,5	1 045,6	1 875,0	1 722,0	83,2	75,2
R21	52,2	2	30	19,8	18,2	963,7	1 212,9	1 546,9	1 316,9	66,2	76,2
R25	59,1	3	32	16,3	16,8	826,1	668,7	1 120,5	1 226,0	58,2	49,0
R6	65,0	1	34	16,6	15,7*	757,2	891,6*	781,3	781,3*	49,9	53,8*

LH = Left uterine horn
RH = Right uterine horn
* = Non-pregnant uterine horn

Table 2: **The mean cross sectional thickness, diameter and number per surface area of the uterine glands of Boer goats from parturition to 34 d post partum**

Goat No.	Days post partum	Thickness of glandular epithelium (μm)		Diameter of glands (μm)		Glands per surface area	
		LH	RH	LH	RH	LH	RH
R24	0,1	29,9	26,0	169,6	151,7	26,3	19,7
R23	0,5	26,6*	32,0	107,5*	118,1	59,3	26,0*
R30	1	33,0	31,7	79,4	80,6	10,0	10,7
R3	1,5	28,6	27,8*	84,5	84,5*	7,3	12,5
R27	2	18,2	20,2	61,7	57,9	33,0	25,0
R13	4	19,8	17,6	62,1	56,0	16,3	18,0
R5	8	21,4	25,9	60,8	57,0	19,3	16,0
R9	12	17,3	22,1	57,3	53,4	15,6	18,7
R10	16	19,2	18,2	51,8	50,9	17,0	23,5
R28	20	20,8	16,6	50,0	42,5	17,3	23,0
R15	24	19,5	16,6	52,9	48,4	56,3	44,0
R17	26	17,9	19,2	59,5	52,2	30,7	74,7
R8	28	17,6	19,5	53,1	54,7	67,8	53,3
R21	30	19,8	21,1	53,1	70,1	33,3	34,3
R25	32	17,3	18,6	51,8	44,8	73,7	47,7
R6	34	16,6	16,6*	41,6	46,1*	92,0	89,0

LH = Left uterine horn
RH = Right uterine horn
* = Non-pregnant uterine horn

caruncular tissue was very prominent throughout the study. The rate of involution could be monitored by the degree of shrinkage of this tissue. At parturition, the proximal area of the maternal villi, which consisted of connective tissue, in which blood vessels were readily visible, was easily distinguishable. The vertical cavities where foetal villi were located could be identified, although no residual foetal villi could be recognised as such.

The cavities between the maternal villi (where the foetal villi were located), rapidly shrunk and were not so readily discernible because the distal area of the maternal villi degenerated into a relatively amorphous mass by 24 h following parturition. At this stage, the cells were in various stages of pycnosis and this process progressed with time. By Day 2 post partum in the caruncular area, the

Table 3: **Values attained by using the equation $y = Ae^{Bt + Ct^2}$ for microscopic histological changes occurring in the post partum uterus of the Boer goat**

Variables y	A*	B**(x 100) ±SE	C ± SE	Day of no change[00]	Value[000] of y at day of no change
Endometrial epithelium (μm)	38,80	-1,3 ± 0,62	-0,00032 ± 0,0002	-20,2	44,18
Lamina propria (μm)	2610,56	-8,7 ± 1,78	0,00172 ± 0,0006	25,2	875,46
Myometrium (μm)	2059,45	5,0 ± 1,58	-0,00212 ± 0,0005	11,8	2759,08
Serosa (μm)	222,65	-5,9 ± 1,30	0,000547 ± 0,0004	53,8	45,85
Glandular epithelium (μm)	26,89	-3,3 ± 0,98	0,000687 ± 0,0003	24,2	18,00
Glands (μm)	95,36	-6,3 ± 1,46	0,00143 ± 0,0005	22,1	47,31
Glands/ surface area	17,55	1,8 ± 2,54	0,00191 ± 0,0008	4,6	16,86

*value at parturition
**percentage in- or decrease in y/day; $c \neq o$
$^{00}t = \frac{-B}{2c}$; $c \neq o$
$^{000}c \neq o$

hyalinised band of connective tissue which formed ventrally to the maternal villi, appeared thicker, more prominent, with a translucent appearance - this is in agreement with findings by Van Wyk[13] in sheep, and he also found that this hyalinised band of connective tissue became thicker and more prominent as time, post partum, progressed. Botha[2] found the arterial and venous blood vessels in this band of connective tissue to be completely constricted by Day 12 post partum. By Day 4, the connective tissue of the maternal villi had degenerated to such an extent that the bases of the villi were no longer identifiable. The epithelium cells of the maternal villi were also not identifiable and the area nearest to the permanent caruncular tissue could only be seen as an amorphous mass at this stage - consisting of connective tissue in which fibroblast cells were present. This necrotic degeneration of the maternal villi progressed, so that by Day 16 post partum, fragments broke loose from the permanent caruncular tissue, which had become even more compact. As the involution process progressed, so the separation between the proliferating connective tissue (ventral to the hyalinised band) and the hyalinised band progressed, until the necrotic maternal tissue and the hyalinised band completely broke loose from the underlying connective tissue and permanent caruncular tissue.

The endometrial epithelium had already started to proliferate from both sides of the intercaruncular area, over the permanent caruncular tissue by Day 12 post partum in the Boer goat, and was 75% complete by Day 24 and completely covered the caruncles by Day 28 post partum. At this stage the permanent caruncular tissue was contracted, with fibroblast cells and fibres compactly arranged - microscopically the uterine involution process in the Boer goat was complete. This stage is reached by Day 30 post partum during the breeding season, and Day 32 to Day 34 post partum outside the normal breeding season in sheep[2] while Van Wyk[13] quotes involution as being microscopically and macroscopically complete by Day 28 post partum, which is in agreement with the time of uterine involution in the Boer goat.

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SEASONAL ABUNDANCE OF TICKS ASSOCIATED WITH INDIGENOUS GOATS ON A NORTHERN TRANSVAAL FARM

Y RECHAV* and C DE JAGER*

ABSTRACT

The 3 most abundant tick species on indigenous goats on a northern Transvaal farm were found to be *Rhipicephalus evertsi evertsi*, *Rhipicephalus appendiculatus* and *Amblyomma hebraeum*. Three other tick species were present in small numbers. The economical and ecological importance of the ticks found on the goats, mainly in rural areas where chemical control of ticks is practically non-existant, is discussed. The high number of goats in the study area and the shift towards alternative methods of tick control, such as the use of resistant hosts, are important factors in the livestock industry of southern Africa.

Key words: Ticks, indigenous goats, northern Transvaal, seasonal control.

Rechav Y.; De Jager C. **Seasonal abundance of ticks associated with indigenous goats on a northern Transvaal farm.** *Journal of the South African Veterinary Association* (1991) 62 No. 1, 10-11 (En.) Department of Biology, Medical University of Southern Africa, 0204 P.O. Medunsa, Republic of South Africa.

INTRODUCTION

The geographical distribution of ticks in South Africa and the hosts on which they feed are relatively well documented[15]. The seasonal abundance of the economically important ticks on cattle has also been recorded from various places in the country[2 11 12 13]. Similar data are available for game animals in different regions of South Africa[4 5 7]. However, less has been published about the seasonal activity of ticks associated with goats[3 8 9 10 11].

This paper reports on the seasonal abundance of ticks on indigenous goats on a farm situated in the northern Transvaal region of South Africa.

MATERIALS AND METHODS

The study was conducted on 120 ha of natural grazing on the farm "Naauwpoort", which is situated in the north western part of the Waterberg mountains (24° 10'S; 28° 20'E) in the northern Transvaal. Acocks[1] defined the vegetation of the area in which the study took place as Sour Bushveld: an open savanna, dominated by *Faurea saligna* and *Acacia caffra*. On some parts of the farm a sourish/mixed Bushveld in which *Acacia caffra* is abundant, is found.

Two seasons, namely a hot and wet summer (October to March) and a cool and dry winter (April to September) occur in the northern Transvaal. The mean maximum and minimum temperatures recorded during winter (July) were 20°C and -4,0°C, and during summer (January and February) 37°C and 14°C, respectively.

Kudu *(Tragelaphus strepsiceros)*, bushbuck *(Tragelaphus scriptus)*, common duiker *(Sylvicapra grimmia)*, steenbok *(Raphicerus campestris)*, caracal *(Felis caracal)* and bushpigs *(Potamochoerus porcus)* are common on the farm. Many small mammals, mainly rodents, are also present in the study area[13].

The 10 goats used during this study had been exposed to ticks on the farm prior to the commencement of the survey. The same animals were used throughout the study, and were not treated with acaricides. The animals were secured and the ticks removed from the various parts of the body. Particular attention was paid to the removal of ticks from around the hooves.

RESULTS

The results are presented as mean number of ticks per goat. The most common ticks found feeding on the goats were *Rhipicephalus evertsi evertsi*, *Rhipicephalus appendiculatus* and *Amblyomma hebraeum*. Other tick species such as *Boophilus decoloratus*, *Hyalomma truncatum* and *Hyalomma marginatum rufipes* were found regularly in small numbers.

Rhipicephalus evertsi evertsi were found in small numbers throughout the year with a peak during mid-summer (November to January) (Fig. 1). Immature stages were removed from the goats in small numbers during early winter (April and May).

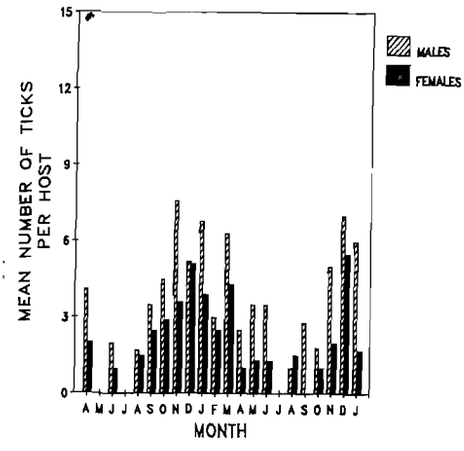

Fig. 1: **Mean number of *R.e. evertsi* adults collected at monthly intervals from ten indigenous goats**

The numbers of adult *Rhipicephalus appendiculatus* increased during October -November, reached a peak later in summer (February) and declined in April (Fig. 2). No adult ticks were found during winter and early summer. Nymphs of *R. appendiculatus* were collected during autumn (April to early June) and early summer (August and September) and were absent during summer and winter (Fig. 2).

Adult *Amblyomma hebraeum* were present on goats during summer and late summer, but absent during winter and early summer (Fig. 3). Unlike the adults, nymphs were present during most of the year with the exception of July. At times

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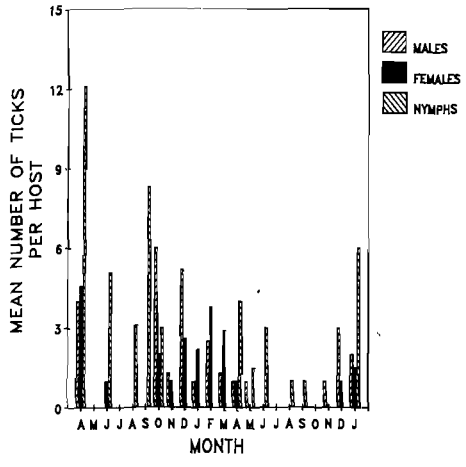

Fig. 2: **Mean number of *R. appendiculatus* adults and nymphs collected at monthly intervals from ten indigenous goats**

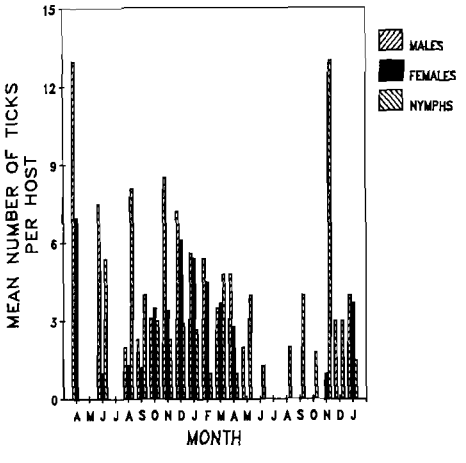

Fig. 3: **Mean number of *A. hebraeum* adults and nymphs collected at monthly intervals from ten indigenous goats**

they were also not found during April and May (Fig. 3).

B. decoloratus adults were found in small numbers during May, September, January and March and were absent during the other months. *H.m rufipes* and *H. truncatum* were also found in small numbers during early and late summer.

DISCUSSION

The common tick species collected from indigenous goats in this survey were *R.e evertsi, R. appendiculatus* and *A. hebraeum*. However, when compared to tick loads on cattle, the density of these species was low[11 13 14]. Our observations showed that the number of ticks carried by the goats mainly during summer was high enough to cause anorexia (resulting in loss of weight or reduction of weight gain) and abscessation[10]. The economical importance of ticks associated with goats is emphasised further by the undeveloped conditions prevailing in the rural areas of northern Transvaal, where chemical control of ticks is practically non-existing.

The role of goats in maintaining tick populations in Natal, has been discussed previously[2]. The high number of goats prevailing in the study area and the fact that they are not treated against ticks has probably resulted in increased numbers of ticks. The shift towards alternative methods of tick control, based on the use of tick-resistant hosts, is an important fact in protecting goats. Preliminary field studies (Rechav 1988, unpublished data) indicated that indigenous goats carried fewer ticks than exotic goats. Exotic goats also failed to acquire resistance against ticks in laboratory experiments conducted in Zambia[6].

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THE PREVALENCE OF THE LARVAL STAGE OF *TAENIA OVIS* AT THE PORT ELIZABETH ABATTOIR

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ABSTRACT

The prevalence of the larval stage of *Taenia ovis* and the origin of the animals were recorded for a period of 39 days at the Port Elizabeth abattoir. Cysticercosis was more frequently found in the *M. triceps brachii* of sheep, but the actual cysticerci were fewer than those found in the semimembranosus, semitendinosus, adductor, biceps femoris, vastus, rectus femoris and gracilis group of muscles of the hind limb (leg muscles). Most animals originated from farms in the Oudtshoorn district.

Key words: *Taenia ovis;* sheep; prevalence; skeletal muscles

Burroughs G.W.; Nel H.M.; Coetzee M.C.; **The prevalence of the larval stage of *Taenia ovis* at the Port Elizabeth abattoir.** *Journal of the South African Veterinarian Association* (1991) 62 No. 1, 12-14 (En.) State Veterinarian, Johannesburg Abattoir, PO Box 86053, City Deep, 2049 Johannesburg, Republic of South Africa.

INTRODUCTION

Cysticercus ovis is the intermediate stage of the tapeworm *Taenia ovis* that occurs in this country in the small intestine of the dog[2 3] and jackal *(Canis mesomelas)* (A. Verster 1988, Faculty of Veterinary Science, University of Pretoria, personal communication). It is sometimes found intramuscularly in sheep and goat carcasses at slaughter and is commonly referred to as a bladderworm[2 3] or muscular cysticercosis[1].

Although the disease has a wide geographic distribution, the incidence is usually low and the clinical effects on sheep and goats are negligible. At abattoirs, however, infested carcasses are condemned or trimmed for aesthetical reasons and often hearts and heads of infested sheep are condemned, which occasionally results in considerable losses to sheep farmers. At the Port Elizabeth abattoir the most common site for the occurrence of the cysticercosis or cysts was found to be the myocardium (Burroughs, unpublished data).

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Bladderworm or muscular cysticercosis occurs in all breeds and sexes of sheep, but young animals are usually more susceptible than adult sheep[1]. Generally muscular cysticercosis is innocuous and affected animals usually show no clinical signs, but very heavy infestations of the heart and skeletal muscles may cause heart failure or weakness in these muscles[1]. At necropsy, the cysticerci are clearly discernible and look like large cysts of *Taenia saginata.* They are found mostly in the myocardium, diaphragmatic crura, masseter muscles, and tongue but other skeletal muscles may also be affected[1].

Until April 1988, several sheep with muscular cysticercosis were found every week and occasionally also a number of goats in the Port Elizabeth abattoir, but no records other than total condemnations of carcasses were kept. In a consignment of 121 lambs, which originated from farms in the Oudtshoorn district, approximately 104 lambs were found to have cysts in the myocardium. The whole batch was detained for secondary meat inspection. In view of the increase in prevalence of the parasite, it was decided to trace the lambs to the district from which they had originated and to determine the distribution of the cysticerci in the carcasses of these lambs.

MATERIALS AND METHODS

Sheep and goats slaughtered at the Port Elizabeth abattoir were routinely examined for cysticerci of *T. ovis.* After April 1988, records were kept for a period of 39 working days, of the origin of 239 sheep and 9 Angora goats with muscular cysticercosis. The carcasses of 19 lambs that were condemned because they were heavily infested with cysticerci, were examined to determine the distribution of the cystercerci in the various muscles. Two additional incisions were made parallel to the routine incision across the *M. triceps brachii* on both sides. Six parallel incisions were made across the fibres of the leg muscles on each side, which included the *gracilis, adductor, semimembranosus, semitendinosus, biceps femoris, rectus* and *vastus* group of muscles. One long incision was made in the *M. longissimus dorsi* and *psoas* muscles on each side. The 1-5 cm pieces of diaphragm that usually remain in the carcasses, were examined by multiple incisions as well as the abdominal muscles and surface of the whole carcass in each case. One incision was made into the breast and shank on each side. The bladderworms were counted and tabulated. The heads and tongues of 75 other sheep where cysticerci were found in the heart, were examined for cysticerci. One incision was made into the *masseter* muscles parallel to the lateral aspect of the mandibular bone. The tongue was examined through a longitudinal incision along the length of the tongue.

Carcasses were passed when no cysticerci could be found on either of 2 deep incisions (one on either side) made across the fibres of the *M. triceps brachii,* similar to incisions made in pigs and cattle when inspecting for cysticercosis, and when all other visible cystercerci could be trimmed away e.g. on the muscles of the abdominal flanks, diaphragm, shanks and those occurring superficially on the carcasses.

RESULTS

During the 39 working days, cysticerci were found in the muscles of 239 sheep and 9 Angora goats. All animals, except for one Angora goat from Malmesbury, originated from the south east Cape, east Cape and Karoo regions (Table 1).

Table 2: **Distribution of the cysticerci of *Taenia ovis* in various muscles of 19 infected lambs**

Lamb No.	Number of cysticerci								
	Leg	Shoulder	Outer surface of carcass	Diaphragm strip	Surface of abdominal muscles	Breast	Shank	M.longissimus dorsi	M. psoas
1	12	4	2	3	3	-	2	2	-
2	21	2	4	3	1	1	-	4	1
3	9	3	1	4	2	-	1	1	-
4	2	2	-	1	-	-	-	2	1
5	5	-	-	1	-	-	2	-	-
6	5	3	-	3	1	1	4	-	-
7	26	2	-	2	2	-	1	-	-
8	4	4	2	3	3	-	2	6	-
9	-	1	2	2	1	-	2	3	2
10	9	1	-	-	-	-	-	1	-
11	4	1	1	3	-	1	-	1	1
12	-	1	-	-	-	-	-	-	-
13	-	2	1	-	-	-	-	-	-
14	14	7	3	3	2	-	3	4	1
15	3	3	3	2	3	-	1	5	-
16	9	3	-	2	3	-	-	-	-
17	5	2	2	1	1	1	2	4	-
18	6	3	-	-	2	-	-	2	-
19	2	3	-	1	2	-	1	2	1
Mean no of cysticerci	7,1	2,5	1,1	2	1,4	0,2	1	2	0,4

Only one cysticercus was found in the masseter muscle of each of 9 heads and two cysticerci in the masseter muscle of one head. None were found in the tongue. The numbers and distribution of cysticerci of *Taenia ovis* in 19 heavily infected carcasses are listed in Table 2.

DISCUSSION

Muscular cysticerci were mostly found in sheep originating from the south east Cape, east Cape and Karoo Regions (Table 1), except for one Angora goat from Malmesbury. The area in question stretches roughly from the Oudtshoorn district in the south to the Fish River in the north and as far inland as Fraserburg and Aliwal North. There was a sudden marked increase in the cases of skeletal muscular cysticerci since good rains had fallen in the areas from where these infested sheep originated (Weather Bureau 1988, personal communication). There were up to 20 cases of cysticercosis per day at that stage. *Taenia* eggs are very susceptible to dessication, but under wet conditions they are known to survive for much longer periods and that probably accounts for the increase in the incidence of muscular cysticercosis (Verster, personal communication).

Although cysticerci were most numerous in the leg muscles (Table 2) cysticerci were most frequently found in the shoulder muscles. Incisions in the *M. triceps brachii* would result in less damage to the carcass than incisions in the leg muscles. The finding of cysticerci on the flank, the surface of the carcass, parietal pleura and peritoneum, diaphragm and psoas muscles show that it is important to examine these sites visually. In 10 out of 75 sheep heads that were examined, cysticerci were found in the masseter muscles and therefore it may be advisable to examine these muscles as well. Examining the tongues of sheep however, appears to be of little advantage.

These results show that in areas where *T. ovis* occurs, the offal of sheep that are slaughtered, as well as the offal and carcasses of animals that die, should be burnt or buried to prevent dogs being infested. Stray dogs and black-backed jackals should be discouraged from frequenting sheep pastures. All dogs on farms should be treated regularly with an effective taeniacide. At abattoirs, Thornton[3] and Gracey[2] recommend that all affected organs should be condemned, while involvement of the skeletal muscle dictates total condemnation of the carcass. USDA regulations state that carcasses with 1 to 5 cysticerci can be trimmed, but those with 6 or more, must be condemned[2,3]. At Port Elizabeth abattoir the inclination has been to follow the recommendations of Thornton[3] and Gracey[2] with total condemnations if skeletal involvement is found. If cysticerci are found in the myocardium, the heart should be condemned and the carcass subjected to a very thorough visual examination. If cysts are found in the abdominal muscles and diaphragm, they should be excised. The skeletal musculature of the carcass should then also be examined as follows:

(a) 3 parallel incisions across the thick part of the *triceps brachii* muscles of each fore limb
(b) one incision through the *gracilis, sartorius, vastus medialis, semimembranosus* and adductor muscles of each hind limb, 2 cm from and roughly parallel to the *symphysis publis* cutting at an angle in the direction of the head of the femur and just missing the *tuber ischii*. These incisions should be carried out on carcasses in a hanging position.

Alternatively, as the larval stage of *T.*

Table 1: **The origin of sheep and goats with muscular cysticercosis slaughtered at Port Elizabeth Abattoir from 9 March 1988 to May 1988**

	Sheep		Angora goats	
	9/3/88 to 14/4/88	15/4/88 to 5/5/88	9/3/88 to 14/4/88	15/4/88 to 5/5/88
Aberdeen	3	11	-	-
Alexandria	2	-	-	-
Aliwal North	1	-	-	
Beaufort West	6	3	-	-
Burgersdorp	1	1	-	-
Cradock	2	6	-	-
Dordrecht	1	-	-	-
Fish River	1	-	-	-
Fort Beaufort	1	-	1	-
Fraserburg	-	1	-	-
Graaff-Reinet	2	3	-	-
Grahamstown	1	3	-	-
Hofmeyer	2	2	-	-
Humansdorp	1	4	1	-
Jansenville	4	5	-	-
Kirkwood	1	1	-	-
Malmesbury	-	-	-	1
Oudtshoorn	-	107	-	-
Pearson	3	-	1	-
Plettenberg Bay	1	-	-	-
Port Elizabeth	4	1	-	-
Somerset East	4	6	3	1
Steytlerville	8	18	-	-
Uitenhage	1	6	-	-
Uniondale	1	1	-	-
Total	53	186	7	2

ovis may be killed by freezing, this measure should be considered as an alternative to condemning all carcasses with muscular cysticercosis. Unfortunately the cysts are larger than cysticeri in cattle and pigs and may be aesthetically objectionable to the consumer. All the cysts in most sheep carcasses with cysticercosis as seen at abattoirs, have already died off and become organised with dense yellowish white connective tissue measuring 3-6 mm in diameter. These degenerated cysts sometimes become infected by bacteria and form abscesses or they may become calcified.

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SOME ASPECTS OF THE LIFE CYCLE OF THE TICK *IXODES PILOSUS* UNDER LABORATORY CONDITIONS

Y RECHAV

ABSTRACT

Some aspects of the life cycle of the tick *Ixodes pilosus* were studied under laboratory conditions. The preoviposition period was 7,2 ± 0,6 d. Maximum egg production was on Day 4 after oviposition commenced, with total egg production of 2 395 ± 128,7 eggs per female. The mean feeding time of larvae was 3,3 ± 0,1 d. The life cycle could not be completed due to the specific requirements of this species.

Key words: *Ixodes pilosus,* laboratory conditions, life cycle.

Y Rechav **Some aspects of the life cycle of the tick *Ixodes pilosus* under laboratory conditions.** *Journal of the South African Veterinary Association* (1991) 62 No. 1, 15-16 (En.) Department of Biology, Medical University of Southern Africa, 0204 Medunsa, South Africa.

Ixodes pilosus Koch is a tick that occurs in the coastal strip of South Africa and Mocambique and in some areas in the northern Transvaal[9]. This tick is common only in areas which have sufficient rainfall to support the growth of long grass[9] and, as in most other African species of the genus *Ixodes,* is restricted to specific humid habitats[3]. *I. pilosus* is of little economic importance, which may account for the absence of any studies on the biology of this species. This report describes some aspects of the life cycle of *I. pilosus* under controlled laboratory conditions.

I. pilosus adults used in this study were collected from cattle in the eastern Cape Province and maintained in a dark incubator at 26 ± 1°C and 80 ± 5% R.H. Preoviposition periods and daily egg production were monitored. Larvae were fed on the backs of naive Himalayan Giant rabbits inside glued containers. Samples of 10 larvae were removed daily from the hosts and weighed.

Engorged larvae were further exposed to relative humidities of 97,5%, 92,5%, 87,0%, 85,0% and 75,0% to stimulate development of nymphs.

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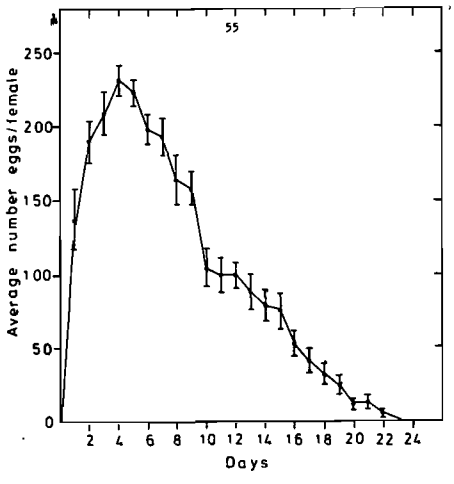

Fig. 1: **Average daily oviposition of *Ixodes pilosus*. Vertical lines represent standard error of the mean**

The mean (±SE) preoviposition period of 20 *I. pilosus* engorged females was 7,2 ± 0,6 d. Maximum egg production was on Day 4 after the commencement of oviposition, with most of the eggs being laid during the first week (Fig. 1). The mean (±SE) daily production was 232 ± 10,3 eggs per female (range 136-314 eggs). The mean (±SE) total egg production (based on 20 females) was 2 345 ± 128,7 eggs per female (range 989 - 2 978). As in many other tick species, a significant positive correlation ($p < 0,01$) was found between the weight of eggs and the weight of engorged females (Fig. 2).

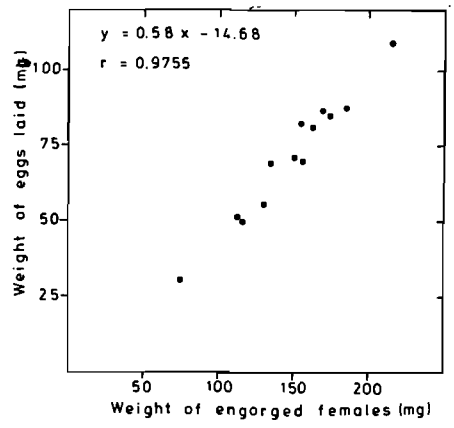

Fig. 2: **Relationship between weight of eggs laid and weight of engorged females of *Ixodes pilosus***

The mean (±SE) incubation period of the eggs was 30,2±3,7 d. The mean (±SE) feeding period of the larvae was 3,3±0,1 d. Weight changes during the feeding period are presented in Fig. 3. Exposure of the engorged larvae to the various relative humidities was unsuccessful because they died of desiccation.

The results from the present study agree

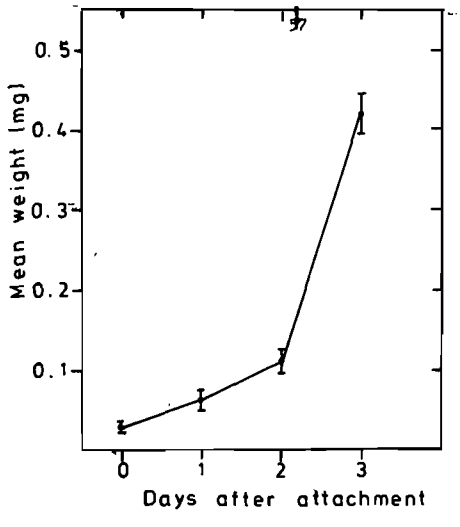

Fig. 3: **The changes in weight of larvae of *ixodes pilosus* during feeding**

with those reported for other African ticks of the genus *Ixodus*[4 5 6 8].

It is necessary to emphasise that *I. pilosus* is very sensitive to desiccation, and found to be abundant mainly in coastal areas with a high rainfall, particularly along densely vegetated riverbanks[9]. This setting provides a very humid microenvironment and enables the development of eggs to larvae to take place. *I. pilosus* laid more eggs than any of the African species of the genus *Ixodes*, with the exception of *I. aulacodi*[6]. Perhaps this is to ensure their survival in such habitats. The requirement of the larvae (and probably also of the other stages) for a very high humidity, may also be the reason for the unsuccessful attempts at breeding *I. pilosus* in the laboratory.

Although the duration of the life cycle of *I. pilosus* could not be completed, it appears that the life cycles of African ticks of the genus *Ixodes*, are generally longer than those of species from temperate zones[1 2 6 7 8 10]. The long life cycle of ticks such as *I. matopi, I. pilosus* and *I. rubicundus,* might regulate the development of these ticks and ensure that the desiccation - sensitive stages of the life cycle occur at favourable times of the year.

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Book review/Boekresensie

POULTRY DISEASES

F T W JORDAN

3rd Edn. Bailliere Tindall, London NW1 7DX, England. 1990 ppXV1 and 497, 85 figures and 15 colour plates. ISBN (0-7020-1339-0).

This book covers the many infectious diseases of poultry caused by bacteria, viruses, mycoplasmas, chlamydia, fungi and parasites. Also covered, are infectious and non-infectious diseases of the musculoskeletal system as well as kidney, cardiovascular diseases, nutritional disorders, some poisons and toxins and various miscellaneous, but nevertheless important conditions, such as swollen head syndrome and infectious stunting syndrome.

Under each disease the aetiology, signs, pathology (both macroscopic and microscopic lesions), pathogenesis, epidemiology, diagnosis and control are dealt with.

The last chapters of this book deal with the correct way to carry out a field investigation, hygiene and disinfection in poultry management, vaccines and vaccinations, antimicrobial medication and welfare matters. There is also an appendix with useful data such as normal haematological values, water and feed consumption, area and feed space requirements, ventilation, lighting and fumigation.

This book is to be recommended as it covers all the important diseases and conditions affecting poultry, is well set out, detailed, up to date and also provides in each section, lists of titles for further reading. Both under- and post-graduate students as well as practitioners in the field, will find this book most useful.

N.M. Duncan

THE EFFECTS OF XYLAZINE AND FENTANYL ON VARIOUS HORMONES AND METABOLITES IN KARAKUL SHEEP AND A BLESBOK

ANNA L MARAIS*, J G VAN DER WALT** and J D SKINNER*

ABSTRACT

Xylazine and fentanyl are commonly used in combinations for immobilisation of wild antelope. In order to ascertain the effects of the combination of these drugs on certain metabolites and hormones in ruminants, blood was sampled from 8 karakul sheep (4 experimental and 4 control) and one tame blesbok (*Damaliscus dorcas phillipsii)* for 30 min before and after immobilisation. The samples were assayed for glucose, free fatty acids, insulin, thyroxine, triidothyronine, progesterone and oestrogen. Significant changes, after the administration of xylazine and fentanyl, were recorded in circulating concentrations of glucose, which increased, and free fatty acids and insulin, which decreased. The other hormones tested were not affected within the sampling period. It is suggested that the combination of xylazine and fentanyl may act directly on pancreatic Beta cells to inhibit the secretion of insulin, which will consequently affect circulating concentrations of glucose and free fatty acids.

Key words: Drugs, metabolites, hormones, sheep

Marais A.L.; Van der Walt J.G.; Skinner J.D. **The effects of xylazine and fentanyl on various hormones and metabolites in Karakul sheep and a blesbok.** *Journal of the South African Veterinary Association* (1991) 62 No. 1, 17-19 (En.) Mammal Research Institute, University of Pretoria, 0002 Pretoria, Republic of South Africa.

Pharmacological immobilisation of free-ranging and captive wildlife has provided a relatively safe and easy way of obtaining blood samples and various measurements for physiological studies. Clarke & Doughton[4] found that although chemical restraining drugs differ widely in physiological side-effects, this method was preferable to manual physical restraint of wild animals. They are thus in agreement with Wesson et al.[18] who reported on the influence of chemical immobilisation and physical restraint on white-tailed deer *(Odocoileus virginianus).*

However, side-effects of drug administration can influence homeostatic mechanisms responsible for maintaining normal serum biochemical and hormone concentrations[7 10 18]. It is therefore imperative to determine possible side-effects before embarking on a physiological study using chemical restraint.

Xylazine hydrochloride has been used for immobilisation of deer (*Capreolus* spp.)[11 17] and combinations of xylazine and ketamine hydrochloride, fentanyl citrate and etorphine for restraint of moose *(Alces alces),* deer *(Cervus elaphus nelsoni)*[15] and various African ungulates[19].

Xylazine is an alpha-2 adrenergic agonist with sedative, analgesic and muscle relaxant properties[9] and is widely used in biomedical research and veterinary medicine. Various studies on dogs (*Canis familiaris)*[8], sheep *(Ovis aries)*[3] springbok *(Antidorcas marsupialis)*[11] and goats *(Capra hircus)*[1 13] conclude that xylazine causes a fasting hyperglycaemia. Garcia-Villar et al.[6], reporting on the pharmacokinetics of this drug, indicated that there were remarkably small interspecific differences in the action of xylazine administered either intramuscularly or intravenously.

Fentanyl citrate is described as a pethidine analogue with pharmacologic actions similar to morphine[2]. This drug is effective for the chemical restraint of large herbivores when mixed with a suitable tranquilliser to counter the respiratory depression caused by fentanyl citrate[19].

No published information was available on the physiological side-effects of xylazine and fentanyl used in combination for immobilisation of wild animals. The present experiment was designed to determine the effects of these drugs on a group of ruminants in order to validate their suitability as pharmacological restraining agents in a physiological study.

Karakul sheep (n = 8) weighing ca 40 kg (4 experimental and 4 control) were trained to stand in metabolic crates and were handled regularly prior to the experiment to minimise sampling stress. Jugular catheters were inserted 24 h prior to the experiment and were filled with heparinised saline to prevent blood clotting. Blood was collected using an automatic suction pump connected to a fraction collector, at a rate of 1 ml min^{-1}, as described[16]. After 40 min collection, an intramuscular injection of a combination of 2,5 mg of xylazine (Rompun, Bayer, SA) and 5 mg of fentanyl (Sublimaze, Janssen Pharmaceutica Pty Ltd), or saline in the case of control animals, was administered. Thereafter, blood was collected for a further 40 min before administration of the narcotic antidote, nalorphine (Lethidrone, Wellcome, SA).

The samples were kept on ice before centrifugation, whereafter they were pooled into 4 pre-injection (A B C D) and 4 post-injection (E F G H) samples of 10 ml each. Free fatty acids (FFA) and glucose were determined on the day of the experiment, and aliquots of the remaining pooled plasma were stored at -20°C awaiting assay for insulin, thyroxine, triidothyronine, progesterone and oestrogen (Diagnostic Products Corporation, Johannesburg, SA). Glucose was determined with the GOD-Perid kit (Boehringer Mannheim (Pty) Ltd, Johannesburg, SA) and the FFA were determined colorimetrically according to a des-

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Received: November 1989 Accepted: October 1990

cribed method[5]. All chemicals used were of analytical grade.

One tame blesbok *(Damaliscus dorcas phillipsii)* was available from which to draw blood samples without being stressed. A jugular catheter was inserted one day prior to the experiment. Blood samples were taken 4 times at 10 min intervals before an intramuscular injection of a combination of xylazine (5 mg) and fentanyl (10 mg) was administered, and 4 times at 10 min intervals thereafter. The samples were treated in the same way as for the sheep samples, and subsequently assayed for insulin, free fatty acids and glucose.

Results were analysed using the Student's t-test.

Circulating serum concentrations of glucose remained constant (~3 mM) in the control animals throughout the sampling period, while in the experimental animals, a significant increase of at least 2 mM ($p < 0,001$) was recorded after the injection of xylazine plus fentanyl (Fig. 1).

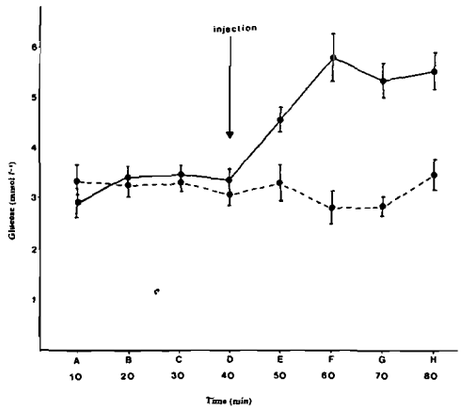

Fig. 1: **Circulating concentrations of glucose (----) before (ABCD) and after (EFGH) injection of xylazine and fentanyl. Control animals (----) received a saline injection**

A significant decrease of almost 0,1 mM ($p < 0,001$) in the serum concentration of free fatty acids was noted after the injection in the experimental animals, while the control animals remained constant at ~0,2 mM. The control animals did not react to the saline injection (Fig. 2).

A significant decrease in serum insulin concentration of over 50 u IU ml^{-1} ($p < 0,001$) was found in the experimental animals after the injection, while concentrations remained constant in the control animals (Fig. 3).

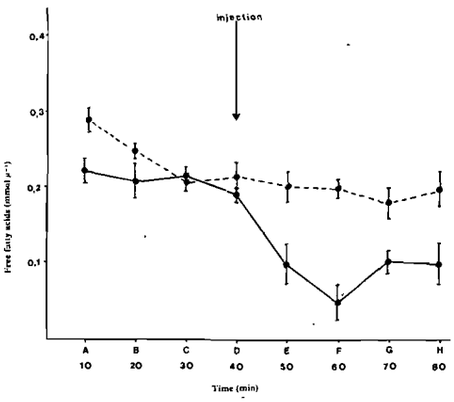

Fig. 2: **Circulating concentrations of free fatty acids (----) before (ABCD) and after (EFGH) an injection of xylazine and fentanyl. Control animals (----) received a saline injection**

No significant changes were recorded in serum concentrations of thyroxine and triidothyronine, either after the administration of the drugs, or between experimental and control animals.

Serum concentrations of progesterone and oestrogen remained constant both before and after drug administration and between control and experimental animals.

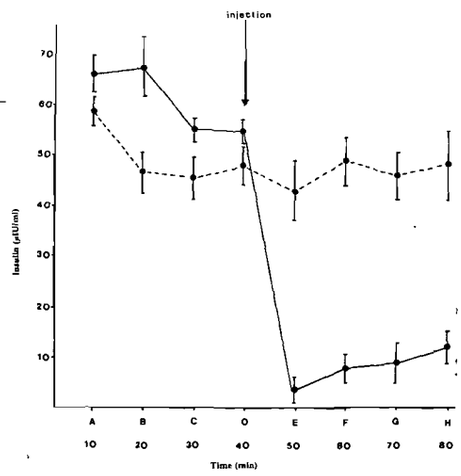

Fig. 3: **Circulating concentrations of insulin (----) before (ABCD) and after (EFGH) an injection of xylazine and fentanyl. Control animals (----) received a saline injection**

Plasma glucose concentrations in the blesbok increased from 1,9 mM to 3,2 mM after the injection of xylazine and fentanyl, while FFA levels dropped from ~0,1 mM to ~0,06 mM. Once again the most dramatic change was the decrease in serum insulin concentrations from ~30 μIU ml^{-1} to ~ 3 μIU ml^{-1}.

The depression of insulin and elevation of glucose concentrations in plasma are typical responses to sympathetic stimulation[3]. Thus, it appears that the hyperglycaemia and hypoinsulinaemia associated with the administration of xylazine and fentanyl may be a sympathetic response mediated via alpha-2 receptors in the brain, rather than those in the pancreas. The present study supports the observations of Nolan et al.[14] that the effect of xylazine on insulin is mediated by the alpha-2 adrenergic receptor.

However, as no significant changes were recorded in hormones secreted by the thyroid or gonads, either before or after immobilisation, it would appear that the combination of xylazine and fentanyl acts directly on receptor sites in the pancreas to inhibit insulin secretion. This supports the hypothesis of Greene et al.[9] that one of the sites of action of xylazine is the pancreatic Beta cell and that the mechanism may involve modulation of calcium transport.

The present results therefore suggest that xylazine and fentanyl should not be used for physiological experiments necessitating pharmacological restraint, where either insulin secretion or plasma glucose and FFA concentrations are critical variables.

ACKNOWLEDGEMENTS

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Book Review/Boekresensie

VETERINARY LABORATORY MEDICINE (Clinical Biochemistry and Haematology)

MORAG G KERR

1st Edn. Blackwell Scientific Publications, Oxford, OX2 OEL. 1989 pp XV111 and 270, 26 figures and 12 colour plates. (ISBN 0-632-02255-8)

There are 3 sections in this book namely those dealing with haematology, clinical biochemistry and practical laboratory medicine.

The section on haematology is subdivided into 3 sections: The section on the erythrocyte which covers red cell parameters; erythropoeisis and the control thereof; erythrocyte morphology; polycythaemia and anaemia. The leucocyte section which covers types, development, counting methods and steroid reaction. The section on platelets and coagulation factors which covers platelet production, function and abnormalities; the coagulation mechanism; anticoagulants and clotting defects and the diagnosis and treatment of bleeding disorders.

The section on clinical biochemistry covers basic principles of biochemistry, the plasma proteins, electrolytes, minerals and nitrogenous substances; carbohydrate, fat and bilirubin metabolism; clinical enzymology; clearance and absorption tests; examination of non-blood body fluids; initial test selection using a standardised approach and individual organ tests.

The section on practical laboratory medicine covers sample collection, processing and storage, the use of external laboratories, establishing and running a laboratory and specific methodologies for haematology and biochemistry.

The book is well laid out and easy to read. The approach is straightforward diagnostic clinical pathology with no frills and exotics added. This book would be most useful to the private practitioner wanting to refresh his/her knowledge in the field of diagnostic clinical pathology or set up a small laboratory in their own practice. For the undergraduate I would recommend this book as supplementary reading.

N.M. Duncan

Book Review/Boekresensie

DISEASES OF CATTLE A MANUAL OF DIAGNOSIS

D C BLOOD, PAULINE BRIGHTLING and M T LARCOMBE

1st Edn. Balliere Tindall, 24-28 Oval Road, London, NW1 7DX. 1991 pp 399, no illustrations, price 9.95 pounds sterling, (ISBN 0-7020-1509-1).

The information in this manual is derived from a cattle diagnostic support computer programme called Bovid. The programme and the manual are cross-referenced to the 1989 seventh edition of the textbook "Veterinary Medicine" by D C Blood and O M Radostits. The manual is divided into 3 parts: a list of clinical syndromes, a list of possible diagnoses for each syndrome and a list of clinical signs for every condition. The user is expected to progress sequentially through each part to arrive at a diagnosis.

There are limitations to the usefulness of this manual which are largely self-imposed by the sub-optimum organisation of the information. The first section has 138 syndromes listed in alphabetical order. The authors accept the constraints of using syndromes instead of clinical signs, but claim that integrating clinical signs to obtain a differential diagnosis list would be impossible. The user is therefore forced to identify a single general syndrome with the possibility of data exclusion. The location of a syndrome within the list can sometimes be difficult to find unless the descriptive term used by the authors is known by the user. Syndromes listed by system may have proved more efficient.

The second section lists conditions which could cause the observed syndrome. These are arranged in descending order of probability. In some cases the list can be enormous — 97 possible causes of acute diarrhoea in yearling cattle are listed. Only the first 15 have been given a probability score. Although not stated in the text, one suspects the probabilities were conceived using Bayes' theorem which has attracted criticism. In addition, the probabilities are only valid for Western Australia and may not be applicable elsewhere. A more general classification into common or rare conditions may have been more useful. An alphabetical arrangement would have permitted condition cross-referencing between syndromes, enabling an inclusive differential diagnosis list to be produced, if more than one syndrome was observed. This would have reduced the amount of data exclusion.

The third section lists clinical signs for over 800 conditions. Each clinical sign has a numerical score which represents the percentage probability that the sign occurs in that diagnosis. The exact meaning of these figures or their origin is not clear from the text. Personal communication with the authors has revealed that the frequencies quoted are point prevalence frequencies, that is, if a veterinarian attended 100 cases, these are the likely frequencies of the clinical signs he/she would expect to see. This assumes a particular stage contact distribution which is not a universal constant and may vary widely depending at which point during the progress of the disease the owner consults the veterinarian. Bovid states that the figures were compiled by a panel of experienced veterinarians and not from case reports. This method can be deceptively inaccurate and caution regarding their reliability may be indicated.

The clinical signs for a condition are arranged randomly which negates cross-referencing between conditions. Listing the clinical signs in descending order of frequency of occurrence would have been an advantage in that the high and low frequency signs could have been expediently identified. Diseases occurring in the tropics are included, but poisonous plants are excluded.

This book suffers from being the by-product of a computer-based programme. Although it is a giant step forward in computer-independent veterinary informatics, it is flawed by the constraints of poor information organisation. The ring binding also makes it susceptible to wear and tear. However, the wealth of information cannot be denied, particularly the clinical sign listings and it is on this basis that I can recommend this book to students and veterinarians involved in the diagnosis of diseases in cattle.

P.D. Cockroft

CARDIOMYOPATHY CAUSED BY AVOCADO *(Persea Americana* Mill) LEAVES

RINA GRANT*, P A BASSON**, HELLEN H BOOKER*, J B HOFHERR*** and M ANTHONISSEN*

ABSTRACT

Six of 21 goats feeding on fresh avocado *(Persea americana)* leaves from pruned trees, showed clinical signs of cardiac distress. Some sheep subsequently dosed experimentally at different dosage rates with the same and other avocado varieties, showed clinical signs of respiratory or cardiac distress and myocardial lesions at autopsy.

Key words: Avocado, *Persea americana,* myocardial lesions, small stock poisoning

Grant R.; Basson P.A.; Booker H.H.; Hofherr J B.; Anthonissen M. **Cardiomyopathy caused by avocado** *(Persea americana* **Mill) leaves.** *Journal of the South African Veterinary Association* (1991) 62 No. 1, 21-22 (En.) Central Veterinary Laboratory, P. Bag X13187, 9000 Windhoek, Namibia.

Twenty-one goats in the Tsumeb district of northern Namibia were fed on freshly cut avocado *(Persea americana* Mill) leaves to supply green fodder during a drought. Three goats died during the subsequent 3 days while another 3 showed signs of general weakness and remained in sternal recumbency. These animals breathed with difficulty at a rate of 28 to 32 cycles per min. Their mucous membranes were slightly cyanotic and their pulses were strong with rates of 84 to 112 per min. All 3 goats had normal rectal temperatures and were anorexic.

Despite the immediate withdrawal of the avocado leaves, all 3 affected goats died within a month. Unfortunately none of the animals that died on the farm were available for post mortem examination. Although the specific variety of avocado could not be identified with certainty, they apparently all originated from saplings of the Fuerte variety with understems of Mexicola. The above-mentioned evidence prompted experimental feeding of the avocado leaves to sheep in order to verify their toxicity.

Leaves stored at 4°C from the original Fuerte variety which poisoned the goats, were only available for one week of the feeding trial (Sheep A and B). Subsequently, leaves from other varieties had to be used for dosing (Sheep C and D). Animals were kept in stables, had free access to water and were supplied with small quantities of lucerne. The fresh avocado leaves were ground and dosed to 4 sheep via rumen fistulae as summarised in Table 1. Blood samples were collected daily from all the experimental sheep. Samples were collected in vacutainers containing EDTA and the following determinations were done on every sample: haematocrit, leucocyte count, differential white cell count, blood urea nitrogen, gamma glutamyltransferase, alanine transaminase and alkaline phosphatase. Autopsies were performed on Sheep A, which died naturally on Day 5, and Sheep B and C which were slaughtered on Day 21 and Day 32 respectively. Sheep D was returned to pasture after being dosed for 31 d.

Specimens of the heart, lung, liver, kidney, spleen, lymph nodes, brain and intestines were preserved in 10% buffered formalin, and processed routinely for sectioning and microscopic study. Sections were stained with haematoxylin and eosin.

A summary of the most pronounced clinical signs is presented in Table 1. An elevated blood urea nitrogen concentration (10,7 mmol ℓ^{-1}), persisted from Day 17 to Day 21 in Sheep B. An arrhythmic heart rate was established in Sheep C at Day 30. In Sheep C, the leukocyte count increased within 2 d of dosing to $> 10 \times 10^9\ \ell^{-1}$ and remained at this level for the rest of the experimental period. It was also observed that the blood sample from this animal took longer to clot than the other samples collected from the other sheep at the same time. Sheep D showed no clinical signs of disease. A leucocytosis was observed one day after the dosage rate was increased to 26g kg^{-1}. The leukocyte count remained above $10 \times 10^9\ \ell^{-1}$ for 17 d and then dropped back to normal.

Microscopic lesions were mainly confined to the heart, and were most pronounced in Sheep A. Cardiac myofibres showed multifocal hydropic degeneration, coagulative Zenker's type necrosis, fragmentation and lysis. The myocardium was intensely congested. The myofibre degeneration and necrosis were occasionally accompanied by a mild neutrophil and macrophage reaction. Cell debris was noticeable in the lumen of the capillaries. Mild changes of a non-specific, subacute, multifocal mixed-cell (neutrophils and macrophages) or mononuclear myocarditis were present in Sheep B and C. Hydropic and fatty changes were present in the livers of Sheep A and B in conjunction with mild nephrosis and mild karyorrhexis of the spleen.

Both circumstantial and experimental evidence suggest that ingestion of large quantities of avocado leaves for more than 3 d could cause mortalities in small stock. It also appears that the toxicity of different varieties of avocado trees may vary, some causing acute lesions which may be fatal, while others merely cause heart lesions without clinical manifestations or might have no effect. Unfortunately the exact strain of the trees involved could not be ascertained.

Kingsbury[1] reported that the leaves, fruit and seeds of avocado trees could be toxic to cattle, goats, rabbits, canaries and fish. Rabbits fed on leaves of Fuerte and Nabal strains died within 24 h, whereas those fed on the Mexicola variety developed no symptoms. The only symptoms described were mastitis and decreased milk production in cattle, and mortalities in goats and canaries which were fed

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Table 1: **Summary of dosage regimens and clinical signs encountered with experimental dosing of avocado leaves to sheep**

Sheep No.	Dose g kg^{-1} day^{-1}	Trial period (d)	Fate	Clinical signs	Macroscopical pathological changes
A*	25	5	Died	Submandibular oedema	Submandibular oedema, ascites, hydropericardium, subepi- and endo cardial haemorrhages. Mild degeneration of liver and kidneys
B*	5,5	21	Slaughtered	Condition poor	Mild ascites, hydropericardium, mild hepatic degeneration
C	2,5	32	Slaughtered	Cardiac arrhythmia	Mild hydropericardium. Mild congestion and oedema of the brain. Degeneration of the myocardium
D	13 25	15 15	Turned to pasture	Slight dyspnoea on Day 15 of dosing	

*These sheep received the suspected poisonous variety

on the fruit. No lesions were reported.

The clinical signs of the natural outbreak, resembled "slangkop" *(Urginea sanguinea)* poisoning in goats in some respects. However, diarrhoea and bloat were absent in the affected animals. The other clinical signs of "slangkop" poisoning in goats such as cardiac arrhythmia, weakness and disinclination to move, dyspnoea and salivation were however noticeable in the sheep poisoned by the avocado leaves.

In conclusion it can be stated that avocado leaves could be toxic to stock, by causing acute heart failure, when large amounts of leaves are consumed for longer than 3 days.

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TREATMENT AND CONTROL OF AN OUTBREAK OF SALMONELLOSIS IN HATCHLING NILE CROCODILES *(CROCODYLUS NILOTICUS)*

K D A HUCHZERMEYER*

ABSTRACT

The therapeutic and managemental steps taken to bring a severe outbreak of salmonellosis in Nile crocodiles *(Crocodylus niloticus)* under control are described. All the crocodiles were initially given intramuscular injections with kanamycin on alternating days for 8 d, coupled with adjustment of the ambient temperature to 29°C. The holding pens were cleaned and disinfected with 2% formalin at the onset of treatment. Daily scrubbing and disinfection was continued throughout the treatment period. Severely affected crocodiles were separated and force-fed a liquid diet. All crocodiles were vaccinated with an inactivated calf paratyphoid vaccine 10 d after the onset of treatment and again one month later. The initial treatment was followed by a 30-week period of in-feed medication with oxytetracycline. Response to the initial treatment was dramatic, although some mortalities still occurred in the force-fed group for one month. The following year's hatchlings were fed heat-treated meat from first feeding onwards to avoid the possibility of introducing *Salmonella* spp. via the feed.

Key words: Crocodiles, *Crocodylus niloticus, Salmonella* spp., control, disinfection, temperature

Huchzermeyer K.D.A. **Treatment and control of an outbreak of salmonellosis in hatchling Nile crocodiles *(Crocodylus niloticus)*.** *Journal of the South African Veterinary Association* (1991) 62 No. 1, 23-25 (En.) Private Practitioner, Lydenburg Veterinary Clinic, Private Bag X20079, 1120 Lydenburg, Republic of South Africa.

INTRODUCTION

Commercial raising of Nile crocodiles *(Crocodylus niloticus)* for the production of leather is an accepted farming practice in South Africa. Problems and management associated with this type of farming are unique and quite different from those encountered with captive crocodilians kept for display purposes.

Being poikilothermic, crocodiles have developed elaborate ways of behavioural thermoregulation centered around the optimal use of energy obtained from food[4]. Free-ranging crocodiles regulate body temperature by temperature selection within thermal gradients in the environment. As with many other poikilotherms, the rate of metabolism and hence growth increases within limits with increasing temperature, provided adequate food is present. Non-feeding crocodilians will seek out cooler temperatures within their environment in order to conserve energy until they feed again. Should a crocodile not be able to avoid high temperatures when not feeding, the situation would be expected to elicit a stress response. Maximum growth is obtained by keeping the crocodiles at constantly high temperatures with daily feeding. The complex behavioural response of thermal selection within an environment of varying temperatures is thus withheld from these crocodiles.

Although it has been demonstrated that alligators show a behavioural fever response towards an infection[4], the immune-defence response appears to increase with temperatures up to a maximum above which it declines. In alligators the leucocyte response to infection reaches a maximum at a body temperature of 30°C. At 35°C body temperature the immune-defence response was rendered ineffective (Glassman & Bennet 1978, quoted by Lang[4]). It therefore appears that the behavioural fever response to infection is limited within the available temperature gradient and once a disease has taken a chronic course with loss of appetite, the animal's response is further complicated by its need to conserve its energy reserves and thus possibly seeking out cooler temperatures.

There have been relatively few documented reports of salmonellosis in farmed crocodiles. The disease appears to be common, with serious economic implications. Foggin[2] reported 70/108 bacterial isolations made from post mortem cases of farmed crocodiles in Zimbabwe to have been *Salmonella* spp.. Friedland[3] also isolated *Salmonella* spp. from septicaemic crocodiles held in intensive rearing units in Lebowa. Contaminated feed, poor hygiene in feed preparation and feeding, stagnant water, and overcrowding resulting in high bacterial concentrations are all regarded as predisposing factors for salmonellosis[1].

Ongoing mortality occurred in a batch of almost 2 000 crocodiles, which hatched on a farm in the Eastern Transvaal in December 1988, soon after they had been put into a closed environment house and had started feeding. Feed consisted mainly of minced meat obtained from feedlot cattle which had died from various causes. According to the farmer, the carcasses had been handled under poor hygienic conditions before arriving at the crocodile farm. Bone meal, carcass meal and a vitamin and mineral premix were added to the minced meat. Bacterial cultures taken at autopsy from a number of these crocodiles, revealed the involvement of several *Salmonella* spp., including *Salmonella typhimurium*. It was suspected that the original source of infection may have been the feedlot carcasses. The farmer treated ill crocodiles with sulphamonomethoxine (Daimeton B, Centaur) and later with gentamycin (Genta 50, Phenix) but with little success. Subsequently, enrofloxacin (Baytril, Bayer) and then sulpha-chloropyrazine (ESB3, Ciba Geigy) were used on the sick crocodiles, both with poor results. All these antibiotic treatments were given at

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far below recognised therapeutic levels and were applied for an inadequate period. In April 1989 an untyped *Salmonella* sp. was again isolated from the sick crocodiles. This isolate had been found to be resistant to the most commonly used antibiotics. An antibiogram however, indicated good sensitivity towards kanamycin, which was subsequently used but was found to reduce mortalities only temporarily. At this stage, the farmer started chlorinating incoming water at 5 ppm chlorine.

Up to 15 crocodiles were dying per day and the appetite of the whole group was severely suppressed despite a constant ambient temperature of 35°C. At this stage the author was consulted. Post mortem examination on 10 crocodiles indicated that the majority of crocodiles probably died of salmonellosis. The purpose of this paper is to document the treatment and management regimens by which the outbreak was brought under control.

MATERIALS AND METHODS

Treatment

Treatment with kanamycin (Kanamyn, Phenix) was continued at 20 mg kg^{-1} body mass by intramuscular injection repeated every 48 h for 8 d. All 1 500 crocodiles still alive, were treated, whether they appeared ill or not.

The weakest crocodiles were sorted into a sick bay where they were force-fed from a syringe on alternating days. A liquid diet consisting of egg, liver and a vitamin and mineral premix was given. The remaining crocodiles were fed as usual during the treatment period. Once crocodiles in the force-fed group appeared to regain strength, they were released back into the pens.

An inactivated calf paratyphoid vaccine (V.R.I. Onderstepoort) was administered by intramuscular injection to all the crocodiles at a dose of 0,2 ml per crocodile 10 d after the onset of treatment. Vaccination was repeated one month later.

Two days after the last kanamycin injection, the crocodiles were put onto a 3-week course of medicated feed containing oxytetracycline at a dose of 75 mg kg^{-1} body mass. This was mixed with the carcass meal, bone meal and vitamin and mineral premix before being added to the minced meat just prior to feeding. At this stage the crocodiles were being fed daily.

Management

Close examination revealed a sticky layer of fat covering the cement surfaces of the holding pens. This feed residue remained, despite daily scrubbing, as no fat solvent was used. After moving the crocodiles, the pens were scrubbed down with 0,1% Teepol (Teepol Orange Concentrated Detergent, Cera Oil S.A.). All the extractor fans and doors were opened and the pens were then scrubbed down with a 2% formalin solution followed by thorough rinsing before the crocodiles were returned. Thereafter the pens were scrubbed daily with Teepol, followed by the addition of 100 ppm iodophore (Adcodyne, Adcock Ingram) to the pen water for a period of one hour. Scrubbing utensils were stored in 200 ppm iodophore. An iodophore footbath was also installed at the entrance to the crocodile house.

The normal ambient temperature of the house was thermostatically controlled and kept constant at 35°C. At the onset of treatment, the thermostat setting was reduced to 29°C. The lower temperature was maintained throughout the initial 8 d treatment period. Culture of one of the *Salmonella* isolates at various temperatures had indicated a distinct drop in growth vigour between temperatures of 29°C and 25°C.

As the most likely source of infection appears to have been the feed, the following year's hatchlings (1989) were fed on heat-treated meat from first feeding onwards. Minced meat was heated to 80°C for a period of 10 min and then allowed to cool slowly. Once cool, the carcass and bone meal and the vitamin and mineral premix were added. The feed was then once again put through a clean mincer which produced a fairly dry pellet.

RESULTS

Response to the initial treatment with kanamycin and the lowered temperature was good, with mortalities dropping to fewer than one per day by the end of the initial 8 d. Mortalities mainly occurred in the group being force-fed. During the 3 weeks that the crocodiles were on in-feed oxytetracycline treatment, mortalities continued to decrease and feed consumption increased dramatically. At no stage was there any indication of an adverse response to either kanamycin or oxytetracycline. The vaccination was also well tolerated. Approximately 50% of force-fed crocodiles recovered and were returned to the pens with the healthier crocodiles. The remainder did not respond and took up to one month to die. Three weeks after the start of the initial treatment, several dead crocodiles were presented for post mortem examination. Severe peritonitis, necrotic enteritis and focal liver necrosis were the outstanding lesions. These crocodiles were severely emaciated and gave the impression of having been chronically ill. A month after the initial treatment, mortalities had become rare and the crocodiles were all eating well and appeared healthy.

The body temperature of the crocodiles was generally found to be approximately 2,5°C lower than the ambient temperature. The reduced ambient temperature therefore resulted in crocodile body temperatures of about 26,5°C.

The hygiene measures were well tolerated by the crocodiles, neither scrubbing nor disinfection having any noticeable adverse effects.

The following year's hatchlings readily accepted heat-treated meat and refused to take raw meat when given the choice 2 weeks later. Growth appeared to be good and by 3 months post hatching, the crocodiles were exceptionally healthy.

DISCUSSION

The pathology caused by salmonellosis in crocodiles is severe and may result in acute mortality. However in many cases the course of the disease may be protracted, affected animals dying from chronic manifestations, such as intestinal occlusion, up to 3 weeks after being identified as ill. This was particularly evident amongst the force-fed crocodiles. The disease may therefore be associated with a protracted period during which the diseased crocodiles also refuse to eat. Nevertheless, in this case the crocodiles had still been kept at the usual constant 35°C of their controlled environment. Environmental temperature was reduced to a level that was more conducive to the effective funtioning of the crocodiles' immune system. It also reduced the stress of a forced high metabolic rate in a non-feeding crocodile.

It appeared that the probable sources of infection were feedlot carcasses infected with *Salmonella* spp.. One would expect a mammalian pathogen to have a temperature preference close to that of its host. The lowering of the ambient temperature in the controlled environment house would have also adversely influenced bacterial growth.

The fairly long course of antibiotic therapy with kanamycin did not appear to impair the renal function of the crocodiles. A prolonged course of oxytetracycline-medicated feed was given to exclude complications by other opportunistic bacteria during the recovery period in the already weakened crocodiles.

High humidity and high temperatures make controlled environment rearing units ideal incubators for bacteria. Although the pens appeared spotless, the sticky fat layer on most of the surfaces of the pens may have provided a rich source of bacterial contamination as well as protection against disinfectants. Although iodophore disinfection after removal of the fat layer is probably not necessary during the normal operation of a closed environment unit, it provides a useful way of effectively disinfecting pens during a disease outbreak.

Once diseased crocodiles show advanced clinical symptoms of salmonellosis (sluggishness and eventually total inabili-

ty to move), the disease has progressed so far that the above measures alone are usually insufficient to ensure their recovery. The high individual value of these animals makes it worthwhile to force-feed the animals in order to supply fluids and to regain a positive energy balance. A survival rate of approximately 50% amongst these crocodiles, where most would have died despite correct antibiotic therapy, indicates a reasonable degree of success.

Whether vaccination had any effect in preventing a recurrence of the outbreak or whether improved hygiene and general health of the population were responsible for the further well-being of the crocodiles is not known. There is no reason to believe that crocodiles would not respond immunologically to inoculation with an antigen.

Crocodiles appear to be particularly susceptible to salmonellosis during the early post-hatching period and it is important not to expose them to *Salmonella* spp. during this period. Meat fed to the crocodiles was heat-treated to exclude the possibility of disseminating *Salmonella* infection. The hatchling crocodiles readily accepted this meat when it was fed immediately after hatching.

CONCLUSION

Antibiotic therapy alone is seldom successful in resolving an infectious bacterial problem in intensively housed populations of poikilothermic animals such as crocodiles and fish. Knowledge of the hosts' preferred environmental temperature while healthy or when diseased, the temperature requirements for maximal immune response as well as the temperature preference of the pathogen are needed to establish a sound treatment regimen. Furthermore, in densely stocked, controlled environment houses, adequate attention must be given to the overall hygiene of the house, particularly during disease outbreaks. Whilst intensive treatment, including force-feeding of individual crocodiles, is time-consuming, it is justifiable due to the high value of the individual animal. Vaccination with a bacterin vaccine is well tolerated and may provide a sound prophylactic measure when dealing with salmonellosis.

ACKNOWLEDGEMENT

The help of Dr. Maryke Henton and her staff, Department of Bacteriology, Veterinary Research Institute, Onderstepoort, is gratefully acknowledged.

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CARNIVORE BEHAVIOR, ECOLOGY, AND EVOLUTION

EDITOR: JOHN J GITTLEMAN

Chapman and Hill Ltd, 11 New Fetter Lane, London EC4P4EE 1989, pp xiv and 620, numerous figures and tables. Price not given
(ISBN 0-412-23350-9).

In sharp contrast to the caring and often sentimental approach of the South African public to dogs and cats, there is a marked ignorance about free-ranging carnivores. Misconceptions about the latter are often fostered by anecdotal accounts of ferocity and cunningness, especially of species like wild dogs, leopards and black-backed jackals. Veterinarians who make a living by providing health care services for dogs and cats, could potentially help to increase public awareness and understanding of the carnivores as a group of mammals. Reading a text like "Carnivore behavior, ecology, and evolution" could provide some of the essential background needed in trying to defend the case of carnivores.

This book presents some of the recent advances in research on selected aspects of carnivore biology. It also highlights existing gaps in our knowledge and in doing so, indicates future research prospects. Following on a general introduction to carnivores, the text is presented in 3 sections, namely behaviour, ecology and evolution. The section on behaviour includes chapters on acoustic communication by fissiped carnivores; the role of odor in the social lives of carnivores; the behavioural development of terrestrial carnivores; the comparative behavioural ecology of hyenas; intraspecific variation in canid social systems; the mating tactics and spacing patterns of solitary carnivores, and carnivore group living. The section on ecology includes contributions on the feeding ecology of giant pandas and Asiatic black bears, adaptions for aquatic living; ecological contraints and predation by large felids; the advantages and disadvantages of small size to weasles; basal rate of metabolism, body size and food habits; and patterns of energy output during reproduction in carnivores. The last section contains contributions on locomotor adaptions by carnivores, carnivore dental adaptions; delayed implantation; molecular and biochemical evolution of the carnivora; the phylogency of the recent carnivora and the fossil history of the terrestrial carnivora.

This work was most likely not compiled with the veterinarian in mind as a possible end-consumer. Most veterinarians would also probably regard most of the information as esoteric in nature. I am of the opinion, however, that veterinarians should never loose sight of the fact that the dog and cat are, in the first instance, carnivores. This should help considerably in an understanding of nutritional and behavioural disorders.

This book is also likely to broaden the outlook of the small animal veterinarian. Instead of simply regarding retrovirus infections in cats as "frustrating hopeless-prognosis" entities, these endogenous chromosomal DNA infections may be perceived as having helped to confirm the monophyletic aspect of the genus *Felis* and the "Domestic cat lineage".

J van Heerden

OBSERVATIONS ON A FIELD OUTBREAK OF POX VIRUS INFECTION IN YOUNG NILE CROCODILES *(CROCODYLUS NILOTICUS)*

F W HUCHZERMEYER*, K D A HUCHZERMEYER** and J F PUTTERILL*

ABSTRACT

A field outbreak of pox virus infection in juvenile Nile crocodiles *(Crocodylus niloticus)*, in which high morbidity and negligible mortality occurred, is described. Histopathological examination of the skin lesions revealed numerous large intracytoplasmic inclusions in the dermis and a very mild dermal inflammatory reaction. Scanning electron microscopical examination of the skin revealed the presence of large numbers of virus particles in the inclusions. Skin lesions persisted for 5 to 6 months.

Key words: Nile crocodile, *Crocodylus niloticus*, pox, stress

Huchzermeyer F.W.; Huchzermeyer K.D.A.; Putterill J.F. **Observations on a field outbreak of pox virus infection in young Nile crocodiles *(Crocodylus niloticus)*** *Journal of the South African Veterinary Association* (1991) 62 No. 1, 27-29 Veterinary Research Institute, 0110 Onderstepoort, Republic of South Africa.

Outbreaks of crocodile pox have been reported from North America[5] and southern Africa[13] and the disease is now stated to be present on many crocodile farms in Zimbabwe and elsewhere in Africa (Foggin C M 1989, personal communication). There are fears that the disease could have serious consequences, not only by causing mortality, but also by reducing the economic value of the survivors as a result of permanent skin damage.

This report describes various aspects of an outbreak of pox infection in hatchling Nile crocodiles *(Crocodylus niloticus)* on a crocodile farm in the Transvaal Lowveld.

A group of approximately 1 500 7-month-old crocodiles, which had hatched on the farm, were housed in a closed-environment unit, situated at least one kilometre away from other crocodile houses and the ponds housing the brood crocodiles. Water for the crocodiles was pumped from wells in a river bed and was treated with chlorine at a rate of 5 mg ℓ^{-1}. In the closed-environment house, the young crocodiles had access to shallow water as well as to dry areas. Air and water temperatures in the unit were maintained at 35°C and 32°C respectively. The crocodiles were fed daily on a mixture of minced red meat, bone meal, carcass meal and a vitamin and electrolyte premix (Soluble vitamins & Electrolytes, Salisbury S.A. Veterinary (Pty) Ltd).

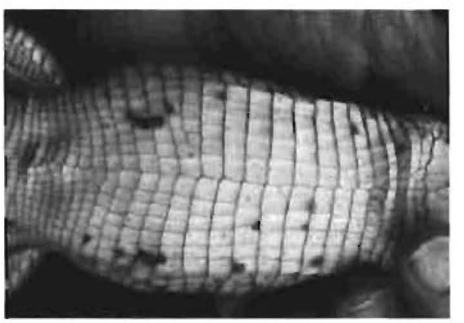

Fig. 1: **Pox lesions on the ventral body surface of a crocodile**

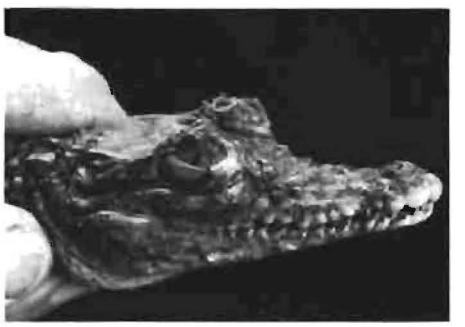

Fig. 2: **Pox lesions on gingivae and eyelids of a 7-month-old crocodile**

The affected crocodiles had survived an outbreak of salmonellosis which occurred 2 to 3 weeks prior to the development of multiple crusty lesions on the skin around the mouths and eyes and on the tail tips. During the outbreak, antibiotic therapy was administered over a period of several weeks, initially by intramuscular injections every 48 h for 10 d, followed by prolonged medication in the feed. This treatment was followed by vaccination of all the crocodiles with an inactivated calf paratyphoid vaccine (Inactivated polyvalent calf-paratyphoid vaccine, Veterinary Research Institute, Onderstepoort). During this entire period, the crocodiles were frequently handled and subjected to the additional stress of daily pond scrubbing and disinfection (Adcodyne, Adcock Ingram).

The skin lesions appeared as dark brown, crusty pox-like lesions up to 3mm in diameter, with a sharply outlined central depression. The lesions were situated between the scales and occurred over the entire body (Fig. 1), but were concentrated mainly on the ventral and lateral surfaces of the body and tail, the upper and lower surfaces of the limbs, and around the jaws and eyes (Fig. 2). Occasionally lesions were evenly spaced in a straight line (Fig. 3). Secondarily infected crusts, which tended to develop into small ulcerated moist areas, were noted in particular on the gingivae around the teeth, on the eyelids and on the feet. The lesions in the mandibular area were often confluent, with an accompanying loss of pigmentation.

The lesions were initially seen in the larger crocodiles within the groups and the latter animals also developed more severe lesions than the smaller crocodiles. During the course of the disease, the number of lesions per crocodile increased steadily over a one-month period and the number of crocodiles affected also increased, resulting in an almost 100% morbidity. The appetite of the crocodiles, which had been severely depressed during

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Fig. 3: **Pox lesions on the lateral surface of the tail of a crocodile. Lesions situated in a straight line (arrow) suggest infected bite wounds**

Fig. 4: **Crocodile pox lesion. Note the inclusions in epidermal cells and mild perivascular inflammatory reaction (arrow) HE X 100**

the salmonellosis outbreak, improved steadily. The occasional mortalities which still occurred, were attributed to chronic lesions associated with salmonellosis (a necrotising enteritis and subsequent intestinal occlusion by fibrinous exudate). Although the pox lesions were numerous on many of the crocodiles, no mortalities could be ascribed to this infection.

Crocodiles with severe facial pox lesions were treated topically with gentian violet (Gentian Violet 1%, Tedro). No other treatment was administered. The health of the crocodiles progressively improved over the next few months, while mortalities virtually came to an end.

When the crocodiles were re-examined 6 months later, they were found to be in good condition and no further mortalities had occurred. Approximately 3% of the population had retained a few faintly visible, focal dark areas on the ventral body surface, presumably at the sites of previous pox lesions.

Crocodiles of approximately 7 months of age (n = 6) with multiple skin lesions from the outbreak described above, were submitted to the Veterinary Research Institute, Onderstepoort. All animals but one, were euthanased and skin lesions excised for freezing in liquid nitrogen for future viral isolation attempts, as well as for light microscopical and scanning electron microscopical (SEM) examination. The remaining animal was kept for further observation.

Frozen sections were prepared from unfixed skin lesions and stained with Sudan IV. Further skin specimens were fixed in 10% buffered formalin, and routinely processed, sectioned and stained with haematoxylin and eosin (HE).

Skin lesions from the body, eyelid and lip of one of the crocodiles were fixed in 4% glutaraldehyde buffered with 0,1M Millonings phosphate buffer (MPB) pH7,3[4] for 24 h. After 2 MPB rinses, the tissue was post-fixed in 1% osmium tetroxide in 0,1M MPB. Two further rinses in MPB preceded conventional ethanol dehydration (50, 70, 90, 96 and 3 x 100%). The samples were then critical point dried (CPD), using liquid CO_2 in a Polaron critical point drier bomb (Bio-Rad, Watford, England). After CPD, each lesion was bisected with a feather-cut razor blade and attached with silver paint (cut surface uppermost) to a SEM viewing stub. The samples were sputter-coated with 30nm of gold using a Balzers SCD 020 Sputter Coater (Balzers Union, Union, Liechtenstein), and then viewed in an Hitachi S-2500 scanning electron microscope at 20kV.

The skin lesions persisted for 5 months on the affected animal which had been kept for observation. One month later, however, only small scars remained on the edges of scales adjacent to previous lesions.

The skin lesions were characterised by marked epithelial proliferation. The epithelial cells were enlarged and filled with single large eosinophilic intracytoplasmic inclusions (Fig. 4) which did not stain with Sudan IV. The perivascular tissue in the dermis adjacent to the epithelial lesions, was infiltrated by a small number of lymphocytes.

Inclusions consisted of densely packed virus particles. These had the appearance of rectangular to ovoid discs measuring approximately 285nm x 195nm x 135nm (Fig. 5-7).

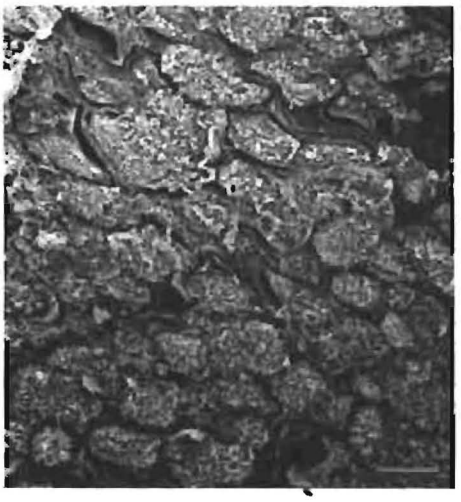

Fig. 5: **Scanning electron micrograph of crocodile pox lesion showing the inclusion bodies (arrow), (bar = 20μm)**

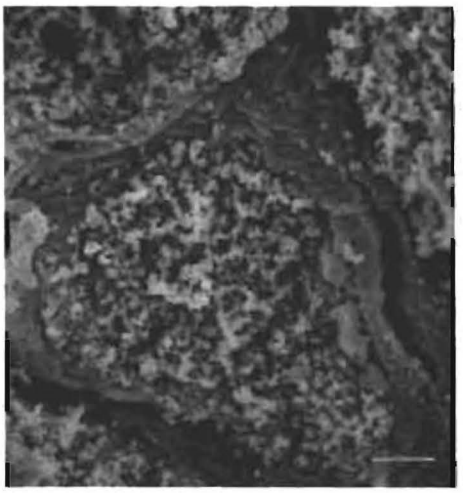

Fig. 6: **Scanning electron micrograph of a single epithelial cell revealing densely packed virus particles within the inclusion body occupying most of the cell (bar = 2 μm)**

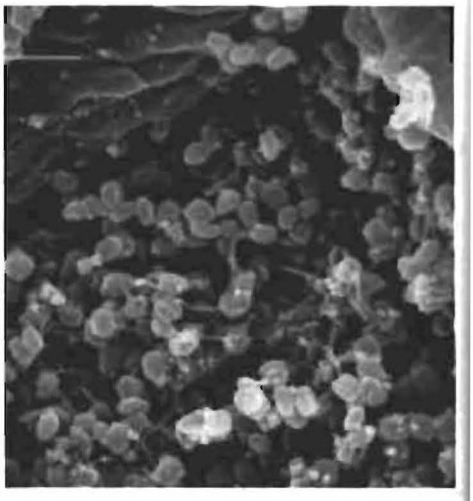

Fig. 7: **High magnification of virus particles in the same cell as shown in Fig. 6 (bar = 0,5 μm)**

Animals infected with crocodile pox in this outbreak, have not yet been slaughtered for commercial use and therefore the effect of the pox lesions on the skins could not be ascertained. However, considering the limited inflam-

matory reaction in the dermis associated with the epidermal lesions, it appears unlikely that the skins would be permanently blemished by scars. The limited dermal inflammatory reaction may furthermore indicate a poor immunological reaction to the infection.

Viral contamination of the environment in a crocodile farm may be very severe as a consequence of the unnaturally dense population of hatchlings. The resulting waterborne infection could then penetrate small skin lesions, including bite wounds as shown in Fig. 3, and consequently cause a large percentage of animals in a pen to become infected.

The epidemiology of crocodile pox infection is as yet unknown. To date, all the reported cases of pox outbreaks have occurred in juvenile crocodiles within one year of hatching[1 3]. In the present outbreak, the animals had been under severe stress. Stress factors were also implicated in the pox outbreak described by Horner[3]. It is likely that wild-caught crocodiles introduced onto most farms as breeding stock, are carriers of the virus. If this is not the case, one would have to postulate the existence of a non-crocodilian virus reservoir. Pox viruses have as yet, however, not been described in any other reptiles or amphibians. While the present outbreak produced minimal mortality, an outbreak in Zimbabwe was associated with heavy losses[1].

Culture of crocodile pox virus, has so far been reported only in crocodile embryo cells (Foggin C M 1989, personal communication). Since crocodile eggs are very expensive and available only for a very short period during the summer, another system or cell line for culturing this virus needs to be found. These difficulties have prevented us up to now from investigating the pathogenesis and epidemiology of the disease. It is also not known, whether recovered animals can act as carriers. Nor is it possible to develop a vaccine to protect animals against this disease without a suitable culture system. The classification of the virus is the subject of a separate study. In HE stained preparations, the inclusion bodies resemble those seen in fowl pox infections. However, the inclusions in the present study did not stain with Sudan IV, which distinguishes this virus from avian pox virus. Furthermore fowl pox lesions are more proliferative and show more marked inflammatory reactions. The recorded measurements of the virus particles in dermal epithelial cells differ only slightly from those of fowlpox virus[6].

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AN OUTBREAK OF BABESIOSIS IN IMPORTED SABLE ANTELOPE *(HIPPOTRAGUS NIGER).*

ELIZABETH F MCINNES*, C G STEWART**, B L PENZHORN*** and D G A MELTZER****

ABSTRACT

A complete necropsy performed on 2 sable antelope *(Hippotragus niger)*, revealed lesions concomitant with a massive haemolytic crisis. These included widespread oedema and anaemia of the carcass, severe oedema of the lungs, petechiae and echymoses of the epicardium, a moderate splenomegaly and a severe haemoglobinuria. The histopathological lesions included a moderate alveolar oedema, the presence of haemosiderin in the spleen and lymph nodes, and mild degenerative changes of the renal tubular epithelium. Peripheral blood and brain smears contained numerous parasitised red blood cells. The parasites were round or oval in shape containing a single or double area of purple-staining chromatin along a portion of the margin of the organism. It was identified as *Babesia irvinesmithi* Martinaglia, 1936, which is unique to sable. Seven sable antelope were subsequently treated with imidocarb diproprionate at a dose of 1,2 mg kg^{-1}. No adverse side-effects have been noted in these animals.

Key words: Sable, *Hippotragus niger,* babesiosis, *Babesia irvinesmithi*

McInnes E.F.; Stewart C.G.; Penzhorn B.L.; Meltzer D.G.A.; **An outbreak of babesiosis in imported sable antelope *(Hippotragus niger)*.** *Journal of the South African Veterinary Association* (1991) 62, No. 1, 30-32 (En.) Department of Pathology, Faculty of Veterinary Science, Medical University of Southern Africa, P.O. Box 176, 0204 Medunsa, Republic of South Africa

INTRODUCTION

Babesiosis in sable antelope *(Hippotragus niger)* has only been recorded on 3 occasions[5] [13] [14]. Martinaglia[5] reported the death, due to babesiosis, of a captive sable antelope, brought from the northern Transvaal to the Johannesburg Zoo. In 1936, Martinaglia named the parasite *Babesia irvinesmithi*[6]. Wilson et al[14] reported the presence of a *Babesia* sp., in the blood smears of 2 young sable that had died of other causes. Thomas et al[13] reported the presence of *Babesia* sp. in 7 out of 124 blood smears taken from sable in South Africa and Zimbabwe. Five of the positive smears were from animals that had been found dead in the veld.

Babesia sp. have been reported from various antelopes. Carmichael & Hobday[2] reported the presence of a small *Babesia* in a tsessebe *(Damaliscus lunatus)* and a large *Babesia* in a blue wildebeest *(Connochaetes taurinus)*. An occasional large *Babesia*-like ring form was seen in impala *(Aepyceros melampus)*[4], while Bigalke et al[1] reported a *Babesia* sp. in a bushbuck *(Tragelaphus scriptus)*.

There is no evidence that *B. bigemina* is harboured by antelope in South Africa[9]. Attempts to transmit the parasite to sable antelope[13], blesbok *(Damaliscus dorcas phillipsi)* and common duiker *(Sylvicarpra grimmia)*[10] were unsuccessful. However other attempts proved the susceptibility of a splenectomised Soemmering's gazelle *(Gazella soemmeringi)* from the Sudan to *B. bigemina* (Enigk & Friedhoff, as cited by Neitz[8]). Attempts to transmit *Babesia bovis* to intact and splenectomised sable antelope were unsuccessful[13].

Nine adult sable antelope were imported from a zoo in West Germany to South Africa. The animals were transported by ship to Cape Town and thence by road to a game farm near Brits in the Transvaal (25° 30' S 27° 48' E). This farm, used previously as a cattle ranch, had been used exclusively for game for the last 4 years. After their arrival, the imported animals were kept in pens on the farm for one month. They were fed silage and lucerne hay and had access to water ad lib. During this time they were exposed to a sable antelope that originated from the Gravelotte district in the eastern Transvaal, which was kept in an adjacent enclosure. This animal had been housed for 2 weeks in one of the pens used for the imported animals 2 months prior to their arrival. No tick control measures were implemented. The 2 imported sable antelope that succumbed, were in the enclosure in which the Gravelotte animal had previously been kept. The 7 surviving sable were housed in individual enclosures adjacent to the above-mentioned pens.

The 2 sable which died, succumbed to the disease approximately 2 months after their arrival in South Africa. Sable 1 was reported to be ill, displaying anorexia, depression and recumbency, approximately 24 h before it died. The carcass was kept at 4°C for 48 h before being submitted for necropsy. Sable 2 did not show clinical signs; it was found dead in the pen, 4 d after the death of the first animal and submitted for necropsy within 12 h. Several *Boophilus decoloratus* and *Rhipicephalus evertsi evertsi* were found on both animals at necropsy.

MATERIALS AND METHODS

A complete necropsy was performed on the 2 sable antelope. Selected tissues were fixed in 10% buffered formalin. Sections of the fixed tissues from these animals were prepared and stained with haematoxylin and eosin (HE). Urine from the bladder was tested for the presence of haemoglobin and protein (N-Multistix, Ames Division, Miles Laboratories Limited Stoke Poges, Slough SL2 4LY England). Peripheral blood smears were made, using blood obtained from the tail. Brain smears were made, using macerated grey matter of the cerbral cortex. An impression smear of the spleen was also pre-

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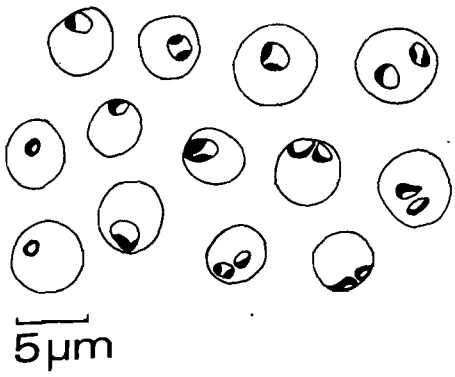

Fig. 1: **Drawings of infected blood cells from Sable 2, showing the different forms of the parasite**

pared. All of the slides were stained with RapiDiff (Clinical Sciences Diagnostics, Division of D.M.S.L. (Pty) Lty. P.O. Box 38939, 2016 Booysens), and examined under a light microscope.

Parasites (n = 100) were measured with an ocular micrometer and mean values were determined. Drawings of the parasites were made with the aid of a drawing tube.

Levels of parasitaemia were determined in the blood and brain smears by counting the number of erythrocytes in one microscopic field and then counting the number of parasites in 15 similar fields.

The 7 remaining imported sable antelope were immobilised using 4-5 mg etorphine hydrochloride (M.99, R & C Pharmaceuticals, Mobani, RSA) together with 40 to 60 mg azaperone (Azaperone, Janssen Pharmaceuticals, Olifantsfontein, RSA) delivered by dart, using Telinject equipment (Telinject SA, Randburg, RSA.). Blood smears were made from each animal with capillary blood obtained from the tail and these were stained and examined as described above. The body mass of each animal was estimated and each was treated with 1,2 mg kg^{-1} imidocarb diproprionate (Forray 65, Coopers Animal Health, Kempton Park, RSA.) administered subcutaneously. No un-toward side-effects were reported to the above treatment.

RESULTS

Post mortem examination revealed that Sable 1 was in a fair body condition, while the body condition of Sable 2 was good. Both the animals had a mild hydrothorax, hydropericardium, ascites and mild pulmonary oedema. In Sable 2, this was evident by the presence of white foam at the bifurcation of the trachea. Both the animals displayed pale buccal mucous membranes which indicated a severe anaemia. Multifocal echymoses and petechiae were present on the epicardial and endocardial surfaces of Sable 2. A moderate enlargement of the spleen was seen in both the animals. The spleen bulged on cut surface and there was an increase in the red pulp. The kidneys of Sable 1 were black in colour, while those of Sable 2 were pale, soft and slightly enlarged.

Both antelope displayed a severe haemoglobinuria and proteinuria. The bone marrow of Sable 2 was pale, yellow, fatty and gelatinous.

The tissues of Sable 1 were too autolysed for histopathological examination. Sable 2 showed severe subendocardial haemorrhage. The lymph nodes revealed severe oedema and focal congestion. Extensive medullary haemosiderosis was present, indicating severe intravascular haemolysis. In addition, moderate erythrophagocytosis was present.

The spleen showed severe congestion of the red pulp with the presence of many macrophages packed with haemosiderin. Despite the presence of parasites in the red blood cells of the brain (as seen in the brain smear), the cerebrum only showed congestion of the blood vessels. No signs of encephalomalacia or haemorrhage were seen. The kidney displayed a moderate interstitial congestion. Mild degenerative changes and small amounts of haemosiderin were present in the tubular epithelial cells.

The pulmonary tissue showed considerable congestion of the interstitial blood vessels. The number of alveolar macrophages was considerably increased and many contained large amounts of haemosiderin. Focal areas of alveolar oedema and interstitial collapse were occasionally seen. In these areas, neutrophils were often present in the interstitium.

The liver showed an increase in the number of neutrophils present within the sinusoids. Haemosiderosis was also evident. A mild periportal inflammatory infiltrate was seen.

The parasitaemia in Sable 1 was 4% and in Sable 2, it was 6%. Extracellular forms were present in both of the antelope. There was a tendency for parasitised erythrocytes to accumulate in the brain capillaries and approximately 40% of these were infected with *Babesia* parasites.

The parasites in the erythrocytes were round, oval, piriform or irregularly shaped, usually with a single or double area of purple staining chromatin situated along a portion of the margin of the organism (see Fig. 1 & 2). The paired forms were usually piriform or irregularly shaped. In Sable 2, there was a tendency for the parasites to occur on the margin of the cell. The round forms in Sable 1 were 0,8-1,8 µm (mean 1,1 µm) in diameter and the elongated parasites measured 2,3-1,0 µm x 1,5-0,8 µm (mean 1,4 x 1,1 µm) while those of Sable 1 measured 2,4-0,8 µm (mean 1,5 µm) and 2,4-1,0 x 1,8-0,8 µm (mean 1,7 x 1,3 µm) respectively. Paired forms were fairly rare.

Examination of the blood smears of the 7 surviving sable did not reveal the presence of any *Babesia* parasites. A *Theileria*-like piroplasm, however, was seen in one smear.

DISCUSSION

Neitz[8] suggested that the relationship between *B. irvinesmithi* Martinaglia, 1936 of the sable antelope and *B. bovis* should be determined. Thomas et al[13] made an attempt to isolate the sable *Babesia* sp. by the subinoculation of blood from sable to splenectomised and intact sable calves and splenectomised cattle as well as transmission with *B. decoloratus* from sable to a splenectomised bovine. Attempts to infect sable with *B. bigemina* and *B. bovis* failed. Thomas et al[13] concluded that *B. irvinesmithi* was a distinct and valid species of sable antelope. Although no transmission studies were completed, we identified the parasite as *B. irvinesmithi*, based on the parasite's morphological characteristics which appear to be identical with those described by Martinaglia[6].

The *Babesia* described in this study is similar in size to that described by Thomas et al[13] The morphology of the parasite from Sable 1 differed in some respects from that of Sable 2. The autolysis in Sable 1 appeared to cause enlargement of the parasite and there were many more round forms present, although oval, piriform and irregular forms were also seen. The mean diameter of the round forms increased from 1,1 µm in Sable 2 (the recently dead sable) to 1,5 µm in Sable 1 (the autolysed sable) and the elongated forms increased from 1,4 x 1,1 to 1,7 x 1,3 µm. Bigalke et al[1] also noted a rounding-off of *Babesia* parasites due to autolytic changes in a bushbuck. Thomas et al[13] observed the parasite to be randomly situated in the erythrocytes. This phenomenon was noted in Sable 1, but in contrast, in Sable 2, 80% of the parasites were situated on the margin of the erythrocytes. These findings suggest that both the size and the situation of the parasite can possibly vary between different specimens. Whether these morphological variations only occur after death cannot, as yet, be determined until more specimens from clinical sable babesiosis are examined.

A comparison of the size of the *B. irvinesmithi* with other cattle *Babesia* (Table 1) shows that the *B. irvinesmithi* is smaller. As these measurements are from relatively fresh material, the increase in size due to autolysis seen in Sable 1 should not be taken into consideration for comparative purposes. This would tend to contradict Thomas et al[13] who suggested a similarity in size to *B. bovis*.

The source of infection for these sable is a matter of speculation. A possibility

Table 1: **A comparison of the sizes of the sable *Babesia* and the bovine *Babesia* species**

Babesia sp.	Authors	Mean Length μm	Mean Width μm
B. irvinesmithi	This study	1,4	1,1
B. irvinesmithi	Thomas et al[13]	1,58	1,11
B. bovis	Gray & de Vos[3]	2,29	1,10
B. bovis	Riek[12]	1,8	1,2
B. bovis	Potgieter[11]	2,0	1,2
B. bovis	Neitz[7]	1,5	
B. occultans	Gray & de Vos[3]	2,88	1,22
B. bigemina	Gray & de Vos[3]	3,29	1,49

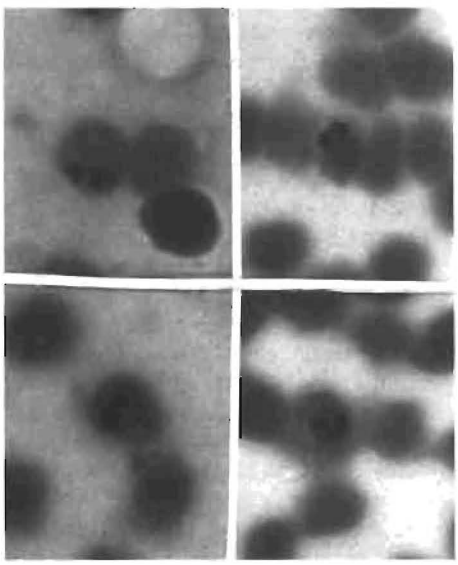

Fig. 2: **Different forms of *Babesia irvinesmithi* from Sable 2 (x100)**

exists that the imported sable relapsed due to the stress of importation. This seems unlikely as babesiosis has not been reported in sable kept in zoos. A less likely explanation is that the parasite was of cattle origin or had originated from another unknown host. Since cattle had not been kept on the game farm for 4 years, and the pens had been recently erected, this possibility seems very unlikely. The most likely source of infection was the Gravelotte sable which had had close contact with these animals.

Wilson et al.[14], reported the macroscopic lesions of anaemia, haemoglobinuria, mild hypoxic lesions, visceral haemosiderosis and sub-pial petechial haemorrhages of the cerebellum found in the necropsies of 2 sable, both of which had *Babesia* parasites in their blood smears. The 2 sable in this study also displayed anaemia and haemoglobinuria at the post mortem examination. Martinaglia[5] reported icterus, anaemia and a splenomegaly in a case of babesiosis in a sable. Moderate enlargement of the spleen was noted in both animals in this study.

The level of *Babesia* parasitaemia is reported to vary between 0,01 and 4,4%[13]. This is similar to the sable in this study which displayed parasitaemias of 4% and 6%, respectively.

Both *B. bovis* and the sable *Babesia* have a tendency to accumulate in the blood capillaries of the brain[13]. This phenomenon was also noted in the brain smears of the 2 sable antelope of this study.

The piroplasm seen in one of the blood smears of the remaining, healthy sable would appear to be a *Theileria* sp.[12]. One of the authors (BLP) found *Theileria* sp. but no *Babesia* sp. in 12/12 clinically normal sable in the Gravelotte area. These findings are in accordance with those of Wilson et al[14], who described theilerial parasitaemias varying from a few parasites to 14,4% in the blood smears of sable calves and adults that they examined; the theilerias did not appear to have an adverse effect on the sable. Similarily, the sable in this study did not display any untoward clinical signs. The findings of Carmichael & Hobday[2] regarding theilerial piroplasms in sable, appear to corroborate the above information.

At present, the importance of babesiosis as a cause of disease in sable is difficult to assess. Wilson et al[14] suggested that latent infections may relapse in animals weakened by other factors and thus contribute to the cause of death. He also speculated that babesiosis may play a role in the high mortality rate of sable calves under the age of 12 weeks. This study shows that if highly susceptible, adult animals, such as those in a zoological gardens situation, which are unlikely to have been exposed to tick infestations, are infected with *Babesia* parasites, then mortality is likely to occur.

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A CASE OF HEARTWORM *(DIROFILARIA IMMITIS)* IN AN IMPORTED DOG AND A REPORT OF THE OCCURRENCE OF CANINE MICROFILARIAE IN THE REPUBLIC OF SOUTH AFRICA

ANNA VERSTER*, W J CILLIERS*** and HEIDI SCHROEDER

ABSTRACT

A dog imported from Australia had microfilaria of *Dirofilaria immitis* in its blood. This is the second report of these microfilariae in dogs imported into the Republic of South Africa. The treatment of this dog is described. Other filarial worms which are known to occur in the RSA are discussed.

Key words: Microfilariae, canines, *Dirofilaria immitis, Dirofilaria repens, Dipetalonema reconditum, Dipetalonema dracunculoides*

Verster A.; Cilliers W.J.; Schroeder H. **A case of heartworm *(Dirofilaria immitis)*, in an imported dog and a report of the occurrence of canine microfilariae in the Republic of South Africa.** *Journal of the South African Veterinary Association* (1991) 62 No. 1, 33-34 (En.) Department of Parasitology, Faculty of Veterinary Science, University of Pretoria, 0110 Onderstepoort, Republic of South Africa

The heartworm, *Dirofilaria immitis,* occurs in the right ventricle and pulmonary artery of the dog and various other carnivores. It has also been recorded in various primates, including man[4]. It is found in temperate, but is more common in tropical countries, such as the Far East, islands in the Pacific, Australia, North and South America; in Europe it is present in Italy, Spain and Portugal[8]. It is not common in Africa, but has been recorded in Kenya[3] and in Malawi[2]. According to Cruz e Silva[1] Travassos Dias recorded *Dirofilaria immitis* in a dog in Beira, Mozambique. It has not been found in dogs bred and reared in the Republic of South Africa (R.S.A.), but microfilariae were found in the blood of a dog imported from Kenya[10].

Numerous species of mosquitoes of the genera *Aedes, Anopheles, Culex* and *Mansonia* which act as intermediate hosts, acquire infection when they take a blood meal. Infective larvae develop in 16 to 20 d and are transmitted to dogs and other definitive hosts when the mosquito feeds on them. In view of the fact that dogs, which are family pets, frequently accompany their owners when they move from one country to another and that this helminth utilises a wide range of mosquitoes as intermediate hosts, it is possible that it could establish itself in the R.S.A.

The purpose of this paper is to report a further positive identification of heartworm and to document the morphological features of other known canine filarids in the R.S.A.

A 5 year-old Alsation crossbred bitch was imported from Brisbane, Australia, on 21 January 1988. According to the owner, she had been or was being treated for a microfilarial infestation prior to being boarded before departure. A health certificate was issued at the same time but no mention was made of *Dirofilaria immitis.* Six months later, she was brought to a veterinary clinic as her behaviour was slightly abnormal and she had not been eating normally for 2 d. Clinically no abnormality was found, but as a blood smear showed a raised eosinophil count, the dog was dosed with nitroscanate (Lopatol, Ciba-Geigy). Blood was collected for examination for microfilaria by Knott's technique (one ml blood is mixed with 9 ml 2% formalin to lake it. Prior to examination, the specimen is contrifuged, the supernatent decanted and the sediment resuspended in distilled water. After centrifugation and decantation the sediment is examined for microfilariae. The specimen may be stained by the addition of a drop of aqueous 1% methylene blue). The identification of the microfilariae as *Dirofilaria immitis* was confirmed by J.R. Georgi (Cornell University, Ithaca, N.Y. USA).

One month later the dog was treated with Ripercol-L (Jansen) (10 mg kg^{-1}) per os daily for 14 d. No microfilariae were subsequently seen in a hanging drop of blood, but microfilariae were present in blood examined by Knott's technique. The dog was then treated with thiacetarsamide sodium (Caparsolate, Abbotts Laboratories), 0,22 ml kg^{-1} live mass twice daily for 2 d by slow intravenous injection. Eight hours after the initial treatment, the dog's lips were swollen and she was treated with antihistamine and prednisolone. Prior to the initial treatment with thiacetarsamide, blood was formalinised for examination by Knott's technique when 240 microfilariae were found in one ml blood. This procedure was repeated on 15 February 1989 when there were 110 microfilariae in one ml blood and the bitch was treated with ivermectin (Ivomec, Logos) at 0,4 mg kg^{-1} per os to destroy the microfilariae. There were no side-effects. No microfilariae were present in formalinised blood collected 2 months after treatment.

The microfilariae reported on in this paper were collected by Knott's technique as described above. The parasites were not stained and were examined with the aid of a compound microscope using 40 x and 100 x objectives. Their length and width were determined by means of a calibrated graticule.

The microfilariae of *Dirofilaria immitis* are unsheathed with a total length of 286-340 μm and a width of 6-7 μm[4]. The anterior end is tapered and the tail is straight[4]. The intermediate hosts of *Dirofilaria repens* are mosquitoes of the genera *Aedes, Anopheles* and *Mansonia.* The adults occur in subcutaneous connective tissue and although they may

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cause nodules, there are often no clinical signs[4]. Autochthonous infections have been found in a dog in Empangeni and a cat in Durban. Microfilariae of this filarid were found in the blood of an imported dog from the United States via Nigeria. (Verster, unpublished data).

The unsheated microfilariae of *Dirofilaria repens* are 268-360 μm long and 5-8 μm wide[4]. This filarid occurs in the subcutaneous tissues in dogs, cats and various other carnivores. It occurs in southern Europe (Italy, France, Spain, Portugal), India, Sri Lanka, south east Asia and the USSR, but not in North America and Australia[4]. Heisch et al.[3] found microfilariae of this species in 2/12 dogs, 19/24 cats and 8/9 genets on Pate Island off the Kenyan coast and according to Levine[4], Van Veen recorded microfilariae in 9% of 188 dogs from northern Nigeria.

The microfilariae of *Dipetalonema reconditum* are unsheathed from 246 to 292 μm long and 5,2 μm wide[4]. According to Sloss & Kemp[7] they are 269-283 μm long and 4,3-4,8 μm wide. Anteriorly the sides are parallel and the tail is curved, like a button hook[4]. Some of these microfilariae have a large, refractile structure (Innenkörper) near the junction of the middle and posterior thirds of the body[4].

The adults of this nematode occur in the subcutaneous connective tissue of dogs[5]. It is present in Europe, North and South America, Hawaii, New Zealand and Kenya[4]. The intermediate hosts of this helminth are fleas, *Ctenocephalides canis* and *Ctenocephalides felis*, and the louse, *Heterodoxus spiniger*[5]. *Dipetalonema reconditum* is non-pathogenic in dogs[4].

In the R.S.A., microfilariae of *Dipetalonema reconditum* are the most common microfilariae seen in the blood of dogs and have been recorded in all 4 provinces. They have also been identified in blood from a dog in Namibia. Van Heerden[9] reported microfilariae of this helminth in the blood of 6 out of 13 captive wild dogs *(Lycaon pictus)*. These microfilariae were also present in the blood of 12/13 wild dogs from the Kruger National Park (Verster, unpublished data). Adult females and one adult male were also recovered from the subcutaneous connective tissue of an animal that died of unknown causes.

The microfilariae of *Dipetalonema dracunculoides* are the smallest ones recovered in this survey. They are unsheathed, 189-230 μm long and 5-6 μm wide. This filarid occurs in the peritoneal cavity of dogs as well as spotted hyaena *(Crocuta crocuta)*, aardwolf *(Proteles cristata)* and the fox *(Vulpes vulpes)* in Africa[4]. It does not appear to be pathogenic. Nelson[6] reported that the louse fly, *Hippobosca longipennis*, was its intermediate host. In the R.S.A., the adults have been found in dogs at necropsy in Vryburg in the Cape Province, and in Windhoek, Namibia. In the Transvaal (Pretoria, Krugersdorp, Rustenburg), adult worms were found in the peritoneal cavity of 3 brown hyaenas *(Hyaena brunnea)*. Verster, unpublished data).

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NON-STEROIDAL ANTI-INFLAMMATORY DRUGS IN DOMESTIC ANIMALS: I. THEIR CLASSIFICATION, MECHANISM OF ACTION AND PHARMACOLOGICAL EFFECTS

G E SWAN*

ABSTRACT

A large number of non-steroidal anti-inflammatory drugs, of different chemical groups are available for veterinary use. These drugs act mainly by inhibiting the formation of endoperoxides (prostaglandins and thromboxanes) through the inhibition of cyclo-oxygenase in the eicosanoid pathway. A wide range of pharmacological effects, including analgesic, antipyretic and anti-inflammatory effects occur as a result of this inhibition. The classification, mechanism of action and pharmacological effects of these drugs are reviewed.

Key words: Non-steroidal anti-inflammatory drugs, review, classification, pharmacology, domestic animals.

Swan, G.E. **Non-steroidal anti-inflammatory drugs in domestic animals: I. Their classifiction, mechanism of action and pharmacological effects.** *Journal of the South African Veterinary Association.* (1991) 62, No. 1, 35-38 (En.) Department of Pharmacology and Toxicology, Faculty of Veterinary Science, University of Pretoria, Private Bag X04, 0110 Onderstepoort, Republic of South Africa.

INTRODUCTION

Non-steroidal anti-inflammatory drugs (NSAIDs) have been defined as those substances, other than steroids which suppress one or more compounds of the inflammatory process[25]. The group is generally restricted only to those substances that act by inhibiting components of the enzyme system in the metabolism of arachidonic acid and formation of eicosanoids[16]. Eicosanoids, which include products such as prostaglandins, prostacyclin, thromboxanes and leukotrienes are a potent group of chemical mediators that play a fundamental role in the inflammatory process[15 30 31].

Salicylates, specifically salicylic acid and sodium salicylate, were the first NSAIDs used in veterinary medicine in the latter part of the nineteenth century[1]. These drugs were found to be "specially servicable in combatting the fever and pain of acute rheumatism"[6]. Acetylsalicylic acid, generically known as aspirin (Disprin, R & C Pharmaceuticals) was introduced in 1889 and was followed by the introduction, of a number of new substituted weak organic acids with basically similar actions and side-effects including phenylbutazone (Equipalazone, Centaur), flunixin meglumine (Finadyne, Centaur), naproxen (Nafasol, Lennon) and meclofenamic acid (Arquel granules, Parke-Davis).

The development of NSAIDs was essentially brought about as a result of the therapeutic limitations of corticosteroids and the search therefore for alternative non-steroidal anti-inflammatory drugs[33]. This search still continues and recently the use of a new non-steroidal anti-inflammatory drug, phenylpyrazoline (BW540C) which exerts both cyclo-oxygenase and lipoxygenase inhibition was reported in the horse[17].

The purpose of this paper is to review and summarise the current knowledge on the classification, mechanism of action and pharmacological effects of these drugs in domestic animals.

CLASSIFICATION

Classical NSAIDs are those drugs which inhibit the cyclo-oxygenase enzyme pathway of arachidonic acid metabolism resulting in anti-inflammatory, analgesic and anti-pyretic effects[15]. However, in future, drugs which inhibit either or both of the cyclo-oxygenase and lipoxygenase pathways in the formation of eicosanoids may also be included. Phenylpyrazolines (eg. BW540C), which are currently being developed are drugs which have broad spectrum inhibition of both pathways[17].

NSAIDs are a heterogenous group of compounds, often chemically unrelated. The main group and subgroups are shown in Fig. 1. Most are substituted organic acids which have been divided into carboxylic and enolic acid groups[25]. Only a few non-acidic NSAIDs exist, including nabumetone and proquamazone[3]. Para-aminophenols although being very weak acids are, however, classified as non-acidic NSAIDs since they have a very large pKa value and would therefore react more like a neutral substance in the body.

MECHANISM OF ACTION

The products of the eicosanoid pathway[34] are responsible for a number of physiological effects. Pharmacologically these effects could be modified or inhibited by specific inhibition of enzymes or neutralization of radicals. The sites at which various drugs can act in the cascade are indicated in blue in Fig. 2. Corticosteroids act by inhibiting phospholipase A[1] and therefore affect both the leukotriene and endoperoxide portions of the pathway. Anti-leukotrienes and dual inhibitors of the cyclo-oxygenase and lipoxygenase enzymes are drugs which are currently under development. Scavengers of free oxygen radicals such as orgoteien, a metallo-protein, prevent the destructive effects of these radicals on cell membranes[1].

Classical NSAIDs act by inhibiting cyclo-oxygenase[16] and thereby prevent the biosynthesis and release of the endoperoxides: prostaglandins (PGE_2, $PGF_{2\alpha}$), prostacycline (PGI_2) and thromboxane (TXA_2) as indicated in red in Fig. 2. Prostaglandins and thromboxanes, as well as other products of the eicosanoid pathway, are part of a physiological control system that is geared to react instantaneously to changes in the homeostasis of organ systems. They affect a wide range of dif-

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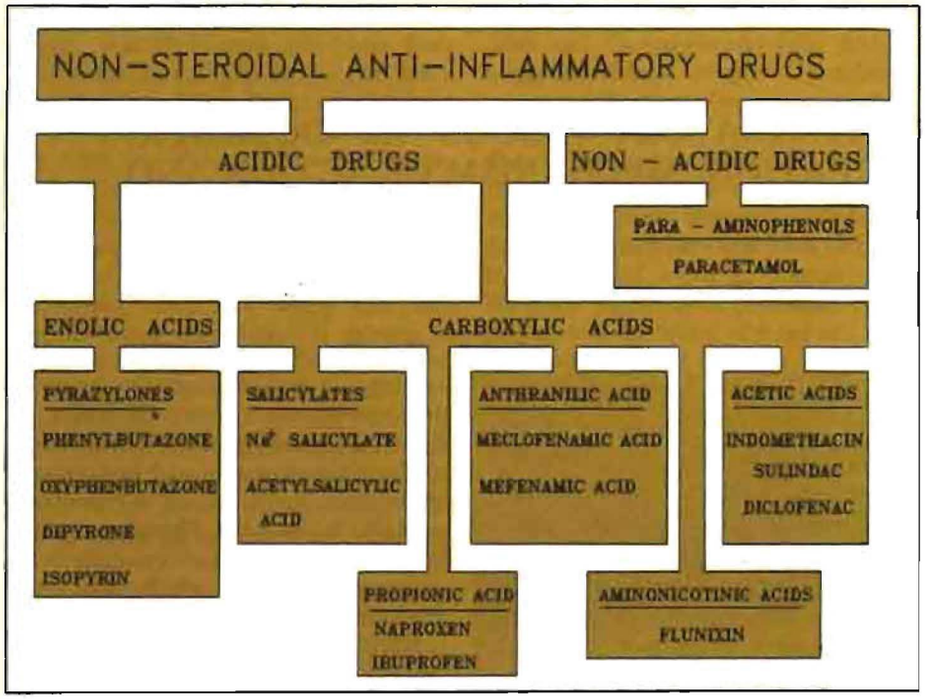

Fig. 1: **Classification of non-steroidal anti-inflammatory drugs (NSAIDs)**

ferent physiological systems with often opposing effects. They are also a group of potent chemical mediators that play a fundamental role in the inflammatory process. Since prostaglandins also potentiate the effects of other inflammatory mediators such as histamine and bradykinin, NSAIDs will also reduce the inflammatory effects of these mediators[37].

Three general types of cyclo-oxygenase inhibition have been described[15 22]. These are reversible competitive inhibition, reversible non-competitive inhibition and irreversible inhibition. Reversible competitive inhibitors include ibuprofen (Brufen, Boots), mefenamic acid (Ponstan, Parke-Davis), some salicylates (Arthridine, Kruger-Med) and indomethacin (Indocid, Logos). The method of inhibition in the case of indomethacin is particularly complex and related to an interaction with the adenylcyclase system by inhibition of phosphodiesterase[15]. Fenemates (mefenamic acid, meclofenamic acid) may also depress prostaglandin actions directly.

Acetaminophen, generically known as paracetamol (Panado, Winthrop), has a reversible non-competitive action, but its action is also partly based on its free radical-trapping properties[22]. It also appears to be more effective against enzymes in the CNS rather than in peripheral tissues. Aspirin, phenylbutazone and flunixin meglumine exert their effects by an irreversible binding to cyclo-oxygenase.

The potency of NSAIDs varies in different animals and this is probably as a result of differences in affinity of the drug for the cyclo-oxygenase enzyme, its effect on the enzyme and differences in plasma binding characteristics. In decreasing potency, in horses the sequences of NSAIDS are: flunixin meglumine, meclofenamic acid, phenylbutazone, naproxen and salicylate[25]. However, potency *per se* does not confer any advantage of one NSAIDs over another. Efficacy is finally determined by the potency versus toxicity ratio.

PHARMACOLOGICAL EFFECTS

Anti-inflammatory

All NSAIDs, except the para-aminophenols have anti-inflammatory activity and provide symptomatic relief of erythema, oedema, fever and pain associated with the acute inflammatory response. Para-aminophenols possess only weak anti-inflammatory activity at the usual dosage rates[22]. NSAIDs exert their anti-anflammatory action by inhibition of the synthesis of cyclo-oxygenase products from arachidonic acid.

The role of eicosanoids in inflammation has recently been reviewed[15]. It is now apparent that members of this group such as PGE_2 and PGI_2 are fundamental to the inflammatory process, particularly in the later stages (3-24 h). Prostaglandins also act synergistically with other mediators such as histamine and bradykinin to potentiate and enhance the inflammatory response[16]. The presence of eicosanoids at the site of inflammation and the persistance or disappearance thereof will therefore affect the progression or resolution of the lesion.

Polymorphonuclear leucocytes are a major source of arachidonic acid metabolites in the acute inflammatory response[19]. Migration of these cells are inhibited by high doses of NSAIDs such as indomethacin, aspirin and flubiprofen[18]. This inhibition was explained by a non-specific inhibition of arachidonic acid peroxidation. Low doses of NSAIDs suppress prostaglandin production but enhance polymorphonuclear leucocyte migration, probably by making more arachidonic substrate available for the uninhibited lipoxygenase pathway[16]. Leukotriene A_4 (LTA_4), which is produced along the lipoxygenase pathway, was found to be one of the most potent endogenous chemotactic factors known[10], and will induce polymorphonuclear leucocyte accumulation.

Analgesia

NSAIDs are effective against pain of low to moderate intensity, particularly pain associated with inflammation or the release of prostaglandins[22]. Prostaglandins alone do not elicit pain but do so in conjunction with other mediators such as histamine and bradykinin[8 9]. Minute quantities of particularly PGE_1, but also PGI_2 have been shown to cause hyperalgesia and to potentiate pain response. Therefore by blocking the formation of these prostaglandins NSAIDs exert their analgesic effect. Several NSAIDs are also effective against kinin-induced pain[22].

Although NSAIDs are generally regarded as being only effective agains pain of somatic and integumental origin flunixin meglumine has been shown tc control visceral pain rapidly and effective ly in equidae[36]. In post operative pair NSAIDs can on occasions be more effec tive than narcotic analgesics eg. ibuprofer vs pentazocine (Sosegon, Winthrop).

In contrast to the weak anti-inflam matory effect of para-aminophenols a normal dosage levels they have gooc systemic analgesic effect. Acetamino phen, the active principle of the para aminophenol analgesics, appears to bc more effective against enzymes in the central nervous system rather than ir peripheral tissues, accounting for its anti pyretic as well as sytemic analgesic ef fect[22]. These drugs are therefore not as ef fective against pain resulting from tissue damage or from the release of inflam matory mediators.

Anti-pyrexia

NSAIDs reset the thermoregulatory areas back towards normal. PGE_1 is a potent pyretic agent[26] and raised concentrations of PGE_2 have been recovered from cerebrospinal fluids of human patients with high body temperatures suffering from a

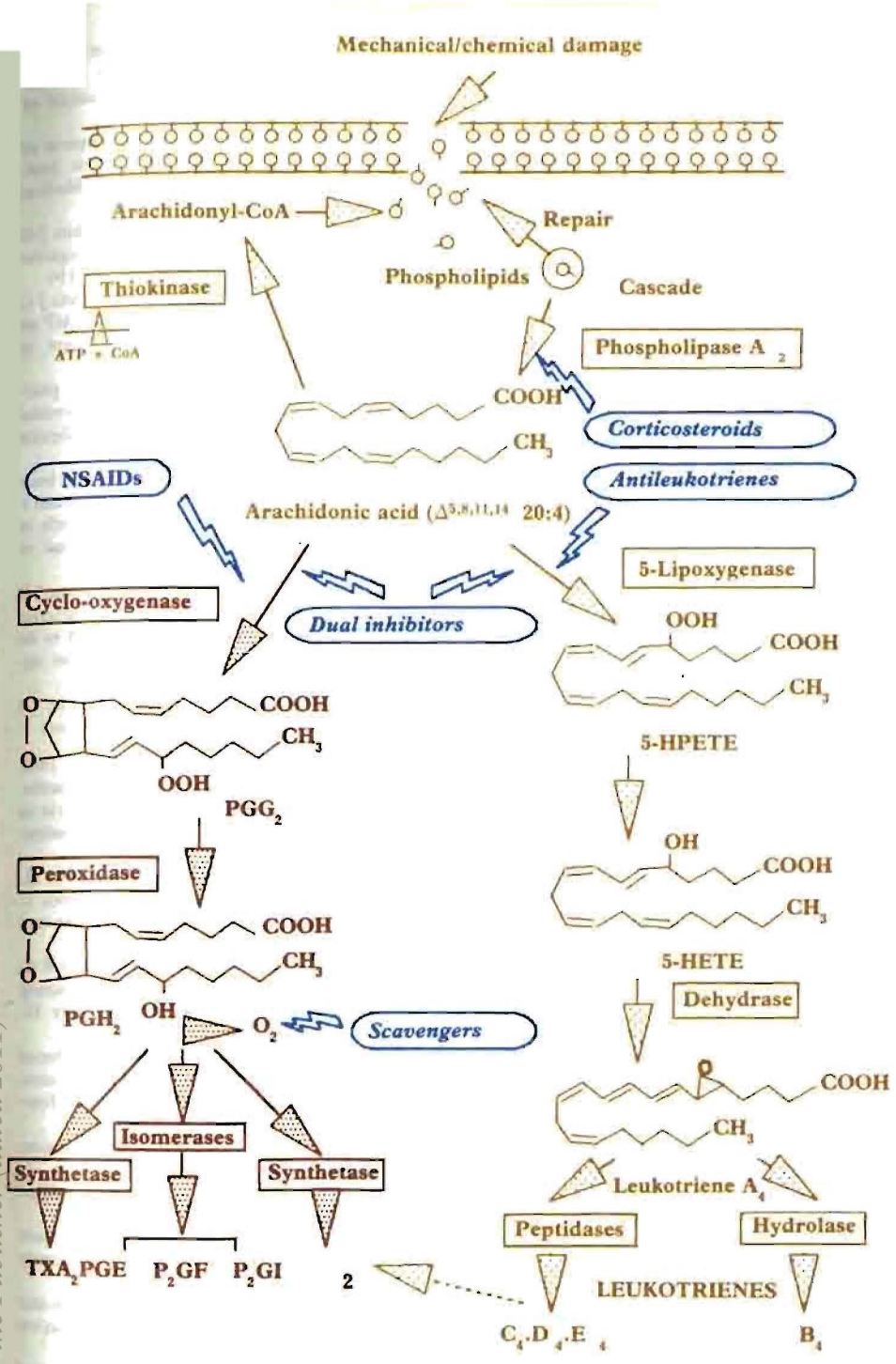

Fig. 2: **Summary of the eicosanoid pathway and sites of action of the various drugs which have an effect on the pathway**

variety of infections, including typhoid and viral encephalitis[32]. The febrile effect of these prostaglandins appears to be as result of a action on the anterior hypothalamus[7]. Blocking the formation of prostaglandins probably accounts for the antipyretic action of many NSAIDs.

Inhibition of platelet aggregation

Aspirin inhibits platelet aggregation by eliminating the release reaction of platelet aggregation induced by adenosine diphosphate and adrenaline. It acts by inhibiting cyclo-oxygenase in the formation of thromboxane A_2[13]. Thromboxane A_2 is a proaggregatory endoperoxide which is metabolised in the platelet from arachidonic acid[14]. Cyclo-oxygenase in platelets is extremely sensitive to inhibition by aspirin and has been estimated as being 20-250 times more sensitive to the inhibitor[4] effects on the enzyme in vascular cells[4]. In contrast to other NSAIDs, aspirin also acts irreversibly in platelets and its inhibition of the cyclo-oxygenase enzyme therefore lasts as long as the platelet survives. Platelets synthetise scant or no new protein. A single ingestion of a therapeutic dose of aspirin thus leads to a platelet defect lasting approximately one week[20].

Prostacycline (PGI_2) also formed along the same cycle-oxygenase pathway possesses the opposite activity on platelets and bloodvessels viz. relaxes bloodvessels and inhibits platelet aggregation[27]. Prostacycline is synthetised in endothelial cells[12] and appears to require at least 10 times more aspirin to completely inhibit its synthesis as compared to the inhibition of platelet cyclo-oxygenase[28]. Prostacycline production furthermore recovers within 6h[21]. Consequently the dose of aspirin has been reduced to selectively inhibit thromboxane A_2 production while preserving prostacycline synthesis[29].

These low doses of aspirin (3-20 mg kg^{-1} in dogs, 25 mg kg^{-1} in cats and 20 mg kg^{-1} in horses) administered once only are effective in inhibiting platelet aggregation for 3-5 d in normal animals of these species[20]. The importance of the platelet endoperoxide pathway differs between and within species[20 23 24].

Pharmacokinetic studies with aspirin provide further support regarding the selective inhibition of thromboxane A_2 production by aspirin. These studies indicate that the irreversible inhibition of cyclo-oxygenase by aspirin occurs predominantly in the pre-systemic circulation[35]. About 60% of the absorbed aspirin is deacetylated to salicylate during the first pass through the liver and the resulting plasma aspirin concentration is probably too low to be associated with any significant cyclo-oxygenase inhibition in systemic tissues, including the vessel wall. Salicylate has a reversible inhibitory effect on cyclo-oxygenase[22].

Uricosuric effect

Salicylate has been shown to alter urate excretion in a number of animals. In man low doses of salicylates decrease renal urate excretion, while in high doses the reverse occurs. Salicylate has little effect on overall urate handling in either Dalmation or other dogs[11].

Phenylbutazone has also been shown to have a uricosuric effect in animals[1]. Sulphapyrasone, a metabolite of phenylbutazone is responsible for the uricosuric effect.

CONCLUSION

Prostaglandins and thromboxanes are responsible for a large number of important and diverse homeostatic physiological functions. Inhibition of their synthesis results in a variety of different pharmacological effects which on the one hand may be used effectively for the treatment of certain conditions such

as inflammation, pyrexia and pain but on the other hand may result in a number of important side effects.

Although the various NSAIDs have the same basic mechanism of action, variation of activity between NSAIDs occur predominantly as result of the type of effect on the cyclo-oxygenase enzyme, due to pharmacokinetic differences and due to other effects such as radical-ion trapping characteristics, effect on the lipoxygenase enzyme and effect on other inflammatory mediators.

The therapeutic indications for use of NSAIDs can be expected to increase in number and diversity as more is understood about the mechanisms by which pain and inflammation is modified[2]. New indications for old drugs are continually being described[5]. Development of drugs with either lipoxygenase inhibition or with dual activity against cyclo-oxygenase and lipoxygenase enzymes will also expand the therapeutic uses of these groups of drugs.

A brief review of the disposition, various therapeutic indications, side and toxic effects and potential drug interaction of NSAIDs is given in part two of this publication.

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