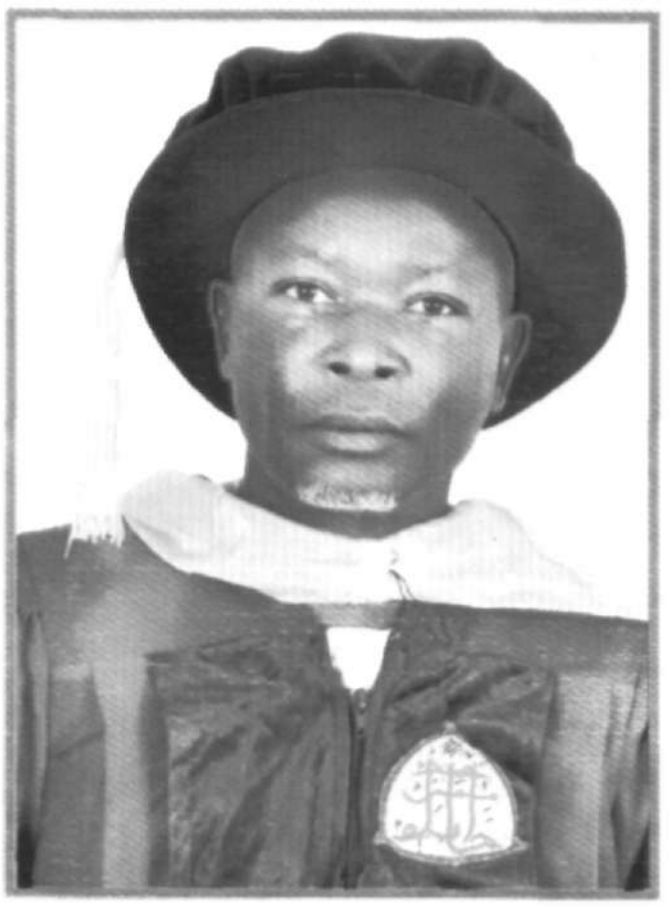


Professor Ibrahim, Najume
Doguwar Giginya
DVM. MSc, Ph.D (ABU), FCVSN
Professor of Veterinary Pathology
Department of Veterinary Pathology
Faculty of Veterinary Medicine

Interconnection of Veterinary Pathology to Applied Medical Sciences

Professor Ibrahim, Najume Doguwar Giginya
DVM, MSc, PhD (ABU), FCVSN
Department of Veterinary Pathology
Faculty of Veterinary Medicine
Ahmadu Bello University, Zaria Nigeria

Inaugural Lecture

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Protocol

- The Vice Chancellor,
- The Deputy Vice Chancellor (Administration),
- The Deputy Vice Chancellor (Academic),
- Other Principal Officers here present,
- Deans, Directors and Heads of Department,
- Patriotic Professors and other Lecturers,
- Non-Teaching Staff,
- Distinguished Guests,
- Our Dear Students,
- Ladies and Gentlemen.

Prologue

Today, I am indeed highly delighted to stand in front of you on such a great and memorable occasion to deliver my inaugural lecture titled; ***Interconnection of Veterinary Pathology to Applied Medical Sciences***. The aim is to cover some of my teaching, research, postgraduate training, routine Veterinary pathological diagnostic activities and community services since the beginning of my carrier as a lecturer in Ahmadu Bello University from 1985 to date.

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Acknowledgement

I am most grateful to Allah the Most Gracious and Most Merciful who enables me to continue working and contributing to the development and progress of our students, the University community and the Nation.

I wish to express my appreciation to the Vice Chancellor, Professor Abdullahi Mustapha, the University authority, the coordinators of the Inaugural Lecture, Ahmadu Bello University, Zaria for the opportunity and the honour given to me to present this Inaugural Lecture titled, ***Interconnection of Veterinary Pathology to Applied Medical Sciences.***

I wish to express my special thanks, appreciation and gratitude to my parents, Alhaji Ibrahim Aliyu, late Hajiya Fatima Ibrahim and the entire members of my family for their support, patience, contributions, understanding and prayers throughout my training. May Allah grant them Aljannatul Firdausi. Amin.
Indeed, words cannot explain my sincere appreciation and gratitude to my able patriotic supervisors throughout my training in this great University, especially Professors C.O. Njoku, K.A.N. Esievo, Dr. P.M. Rahamathulla, Professors D. I. Saror and Y. O. Aliyu for their supervision, constructive scientific criticisms in the course of my training at different stages that led to successful completion of my academic programmes. I acknowledge my senior Professors, Colleagues and other Lecturers for their support and cooperation in teaching, collaborative researches, postgraduate and individual researches which I supervised and/or co-supervised. The contributions of the entire academic staff of the Department of Veterinary Pathology and Microbiology now Departments of Veterinary Pathology; Veterinary Microbiology and the Faculty of Veterinary Medicine are acknowledged. I thank you all including those that could not be listed due to time constraint.

Introduction

According to Stedman's Medical Dictionary as cited by McGavin and Zachary (2007) "Pathology is the medical science, and specialty practice, concerned with all aspects of disease, but with special reference to the essential nature of abnormal conditions as well as the structural and functional changes that result from the disease processes."

It is this particular aspect of science that I have been teaching, training postgraduate students, researching and carrying out diagnostic activities since August, 1985 (29 years) when I was employed as an academic staff by Ahmadu Bello University.
It is my pleasure today to stand before you to discuss some of my contributions to knowledge and to humanity.

The activities I am going to discuss were essentially towards general improvement of animal and human health, particularly under the following headings:

1. *Toxicologic pathology*
2. Food security
3. Experimental pathology
4. Routine diagnostic pathology services to our immediate community and referred cases

1. Toxicologic Pathology on:

(A) Screening of Kargasok Tea: Studies on Pathological Effects in Balb/C Mice And Wister Rat

Kargasok tea was a popular beverage prepared by yeast fermentation of a tea-sugar solution. It's origin was generally believed to have been from the then Union Socialist Soviet Republic [USSR] when a female tourist visited Kargasok in Siberia in 1982 were she found unusually large number of people of over 100 years of age, in good health and with hardly

any wrinkles (Ajayi. 1988a.b; Anon, 1988).This was attributed to their drinking of 300 ml/day of the yeast fermented tea-sugar solution. The tea spread to Japan, Taiwan, Hong Kong and many other countries in the world including Nigeria, Ghana and other West African Countries in 1988 (Aideloje, 1988; Anon, 1988). For two years Kargasok tea enjoyed wide patronage in Nigeria (Akinwade, 1988). As a result of its many curative claims, the tea was nicknamed; "Wonder tea" (Akinwade, 1988), "Tea of life", "Magical tea" and "Treasure tea" (Aideloje, 1988).

Since 1990, the use of Kargasok tea in Nigeria was discouraged by the then Honourable Minister of Health, Prof. Olukoye Ransome-Kuti in a nationwide broadcast and in press conferences by the Directors of Medical and Pharmaceutical Research Institutes in the country.

The discouragement was based on the fact that no scientific evidence was available to ascertain most of the claimed medicinal attributes of the tea and because its effects when used for short and long term were largely unknown.

Against this background, the National Institute for Pharmaceutical Research, Abuja funded this project for us to screen Kargasok tea for its possible beneficial and/or harmful effects to substantiate or refute its many medicinal claims in laboratory animal models as is the case with all new drugs.

At the end of the experiment in which 48 BALB/C mice (30 males, 18 females) and 18 male Wistar rats were given Kargasok tea orally in their drinking water. Zero, 15, 25, 50, 75 or 100 % (w/v) concentrations were administered to mice for 2 or 8 weeks, while the rats were given 0, 15 or 50% concentrations of the beverage for 12 weeks. The results showed that neither the mice nor the rats' organ/body weight ratios were significantly affected by the treatment (Tables 1 and 2).

Table 1: Organ/Body Weight Ratios (%) For Control and Test BALB/C Mice on Kargasok Tea

Organs	**0% KT**	**50% KT**	**100% KT**
Stomach	0.810 ± 0.108	0.945 ± 0.197	1.032 ± 0.099
Liver	4.310 ± 0.128	4.153 ± 0.175	4.893 ± 0.145
Spleen	0.424 ± 0.037	0.445 ± 0.020	0.475 ± 0.080
Lungs	1.062 ± 0.093	1.095 ± 0.049	1.269 ± 0.112
Heart	0.447 ± 0.074	0.368 ± 0.021	0.396 ± 0.040
Brain	1.344 ± 0.112	1.601 ± 0.079	1.519 ± 0.193
Kidneys	1.527 ± 0.130	1.290 ± 0.181	1.396 ± 0.176
Testes	N D	0.653 ± 0.008	1.114 ± 0.012
Uterus	0.336 ± 0.036	0.423 ± 0.007	0.523 ± 0.011

Data are mean ± SE

* Kargasok tea (KT) was administered over a period of 8 weeks.

In all instances, P> 0.05 relative to the control (Student's t-test).

ND: Not determined.

N = 8 per group.

Table 2: Organ/Body Weight Ratios (%) For Control and Test Wistar Rats on Kargsok Tea

Organ	0% KT	15% KT	50% KT
Stomach	0.372 ± 0.006	0.391 ± 0.011	0.390 ± 0.023
Small Intestine**	0.101 ± 0.015	0.092 ± 0.007	0.094 ± 0.008
Large Intestine**	0.122 ± 0.012	0.102 ± 0.007	0.100 ± 0.006
Liver	3.280 ± 0.072	3.300 ± 0.059	3.290 ± 0.092
Spleen	0.246 ± 0.015	0.265 ± 0.019	0.310 ± 0.028
Lungs	0.548 ± 0.028	0.607 ± 0.033	0.604 ± 0.031
Heart	0.282 ± 0.014	0.272 ± 0.014	0.286 ± 0.015
Brain	0.670 ± 0.034	0.773± 0.041	0.773 ± 0.053
Kidneys	0.561 ± 0.016	0.580 ± 0.008	0.543 ± 0.017
Testes	0.891 ± 0.035	0.888 ± 0.038	0.946 ± 0.034
Epididymis	0.392 ± 0.019	0.401 ± 0.019	0.385 ± 0.017
Seminal Vesicles	0.393 ± 0.062	0.313 ± 0.025	0.328 ± 0.023

Data are mean ± SE

* Kargasok tea (KT) was administered over a period of 12 weeks.

In all instances, P> 0.05 relative to the control (Student's t-test).

N = 8 per group.

Table 3: Histopathological Lesions Due To Kargasok Tea Administration to BALB/C Mice

Organs	0%	15%	25%	50%	75%	100%
Brain	-	-	-	-	-	-
Stomach	-	-	-	-	-	-
Intestine (Ileum and colon)	-	-	N	-	-	-
Pancreas	N	-	N	N	N	N
Liver	-	-	-	-	-	-
Spleen	-	-	-	-	-	-
Trachea	N	N	-	N	N	N
Lung	-	-	-	-	-	-
Heart	-	-	-	-	-	-
Testis	N	-	-	-	-	-
Uterus	-	-	-	-	-	-
Ovary	N	-	N	-	N	N
Kidney	-	-	-	-	-	-

N = Not taken – = No significant findings

Table 4: Histopathological Lesions Due To Kargasok Tea Administration To Wistar Rats

Organs	0%	15%	50%
Brain	-	-	-
Stomach	-	-	-
Duodenum	N	+	++
Pancreas	N	+	++
Intestine (Ileum and Colon)	-	+	+
Liver	-	+	+
Spleen	-	-	-
Lung	-	-	-
Heart	-	-	-
Testis	-	-	-
Epididynis	-	-	-
Seminar vesicle	-	N	-
Kidney	-	-	++

N = Not taken – = No significant findings
+ = Lesions were found ++ = More severe lesions were found

The rats that received 15 or 50% concentrations had non-suppurative necrosis of the duodenum, pancreas and intestine (Plates 1, 2 and 3) and nephropathy (Plate 4). No significant microscopic lesions were found in any of the treated mice even at 100% concentration of the tea.

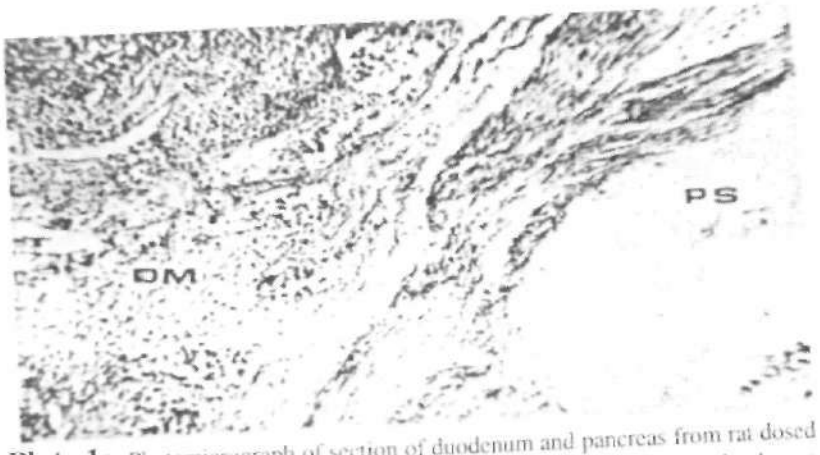


Plate 1: Photomicrograph of section of duodenum and pancreas from rat dosed with Kargasok tea. Note: Non-suppurative necrosis of the duodenum (DM) and pancreas (PS). H and E stain. X 448.



Plate2: Photomicrograph of section from the duodenum (DM) and pancreas of rat dosed with Kargasok tea. Note: Non-suppurative necrosis of pancreas (PS) H and E stain. X 448



Plate 3: Photomicrograph of section from intestine of rat dosed with Kargasok tea. Note: Non-suppurative necrosis of the ileum H and E stain. X 256



Plate 4: Photomicrograph of section from kidney of rat dosed with Kargasok tea. Note: Non-suppurative necrosis of the proximal (P) and distal (D) convoluted tubules, atrophied glomeruli (AG) and congested blood vessel (C). H and E stain. X 448

These findings suggest species variation in susceptibility to toxicity from the tea (Tables 3 and 4). It indicated that the tea may be a cause of renal failure, diabetes mellitus and malabsorption syndrome in the intestine and therefore the tea could not be safe for human or animal consumption (Ibrahim and Njoku, 1991; 1993).

(b) Toxicity Studies on Clinicopathological Effects of Water Extract of Herb 25 -An Antimalarial Herb

As part of the efforts to determine the safety of Herb 25 when used as an anti-malarial preparation, clinicopathological studies were carried out in apparently normal healthy rats orally administered water extract of Herb 25 according to the experimental protocol.

Materials and Methods

Experimental Animals

Eighty-two apparently normal and healthy rats were purchased from the National Research Institute for Chemical Technology (NARICT), Basawa, Zaria. The rats were transported in cages in a roofed but highly ventilated vehicle to the then Department of Veterinary Pathology and Microbiology, Faculty of Veterinary Medicine, Ahmadu Bello University, Zaria, the venue of this research work. The rats were accommodated in a well ventilated animal house and fed on pellated feed prepared from standard poultry growers' mash. Water was provided to the rats *ad libitum*. A period of 3 weeks was allowed for the rats to adequately acclimatize to the new environment prior to the commencement of the experiment. During this period of acclimatization, the rats were made to become accustomed to the different routine handlings they would be subjected to during the experiment, such as simple restraints, taking rectal temperature, body weight determinations and administration of

water orally using insulin syringe in which the needle has been removed. National Institute of Health (NIH) guidelines (NIH, 2002) on the care and use of laboratory animals were strictly followed. Twelve rats were kept as stand by, but, they were finally not used since there was no mortality.

Animal Allocation and Reconstitution of Herb 25 Water Extracts

A total amount of 225g of Herb 25 water extract was dissolved in exactly 225ml of distilled water to obtain a 100% w/v concentration of the herb. The preparation was stored at refrigeration temperature until required. A day prior to the commencement of the experiment (i.e. administration of Herb 25), the rats were allocated to seven groups, A1, B1, C1, A2, B2, C2, and D, consisting of 10 rats each. The rats in groups A1, B1, C1, A2, B2, and C2 were the treatment experimental animals while group D rats served as control. The rats in each group were marked and labelled using roman numerals I-X. The weights in grams, of each of these rats were taken using electronic balance and the values were recorded.

Oral Treatment of Rats with Herb 25 Water Extracts

On each day of treatment, the requisite volumes of the preparation was dispensed into clean containers and allowed to warm to room temperature before administering to each of the experimental animals. Each of the rats in group A1 was orally administered 0.21ml of the preparation that contained 0.21g of the herb 25 water extract in the morning between 6.00am and 6.30am, another 0.21ml of the preparation was administered via the same route to each of these rats in the evening between 6.00pm and 6.30pm.The drug regimen was carried out for five consecutive days. The rats in groups B1 and C1 received similar treatments on the same days of treatment of the rats in group A1 but , such treatments were carried out for seven and

ten days, respectively. Each of the rats in group A2 was orally administered 0.27ml of the herb preparation containing 0.27g of the herb 25 water extract in the morning between 6.00am to 6.30am and 6.00pm to 6.30pm for five days while 0.27ml of the preparation was administered to each of the rats in groups B2 and C2 via the same route of treatments as in group A2 rats, but for seven and ten days respectively. Each of the rats in group D (the control) was administered 0.27ml of portable water via the same route for ten days.

Clinical Observations

Beginning from the day of the commencement of the treatment of the rats with Herb 25, tagged day 0 of treatment, the rats were closely monitored for clinical changes that could be consequent to the treatment with Herb 25 water extract.

The weights of each of the rats were measured and recorded accordingly. The rats in the same group were then humanely sacrificed by first anaesthetizing them with ether before severing their jugular veins. Sufficient volume of blood sample was collected from each of the rats in the treatment and control groups at the time of sacrifice and used for haematological analyses. The haematological values were determined according to the techniques described by Coles (1974), it included packed cell volume (PCV), total white blood cells (WBC) and differential white blood cell counts. Postmortem examination was carried out on each of the sacrificed rats in the treatment and control groups for presence of gross lesions. Tissue samples from various organs of the body were taken and fixed in 10% buffered neutral formalin. The tissues were dehydrated with graded series of alcohol and embedded in molten paraffin wax. They were sectioned at 6µm using a microtome knife attached to a rotary microtome. The sectioned tissues were mounted on clean grease-free slides smeared with egg albumin. The slides were stained with haematoxylin and

eosin stains, cover slipped and dried in oven (Drury *et al.*, 1993).

Result

Clinical observation findings

All the experimental rats in the various groups were alert and healthy. There was no abnormal behavior or neurological changes in any of the treated rats throughout the experimental periods. They had increased appetite. There was no mortality in any of the groups that were treated with different doses (0.42g and 0.54g per day per rat) of the water extract of Herb 25 throughout the different experimental periods (5 days, 7 days and 10 days).

Weights Pre and Post Treatment with Herb 25 Water Extracts

The weights of the rats before and at the end of treatment with Herb 25 were as presented in table 1 below. The values indicated that the rats gained weights during the experiment. Apparently, the treatment with the water extract of Herb 25 did not retard weight gain. On average, the rats in the groups A1, B1, A2, C2, and D had gained weight by about 5%, 7.1%, 3.9%, 10.3%, 14.8%, 7.7%, and 7.1% respectively (Table 1).

Table 1: Weights (g) of experimental rats pre- (1) and post- (+) treatment with the water extract of Herb 25

Anim No.	A_1(-)	A_1(+)	A_2(-)	A_2(+)	B_1(-)	B_1(+)	B_2(-)	B_2(+)	C_1(-)	C_1(+)	C_2(-)	C_2(+)	D(-)	D(+)
I	136	166	187	206	176	179	207	222	106	142	147	155	173	178
II	197	200	171	171	153	149	163	193	160	197	181	183	209	214
III	187	193	130	146	164	179	184	204	200	203	149	157	103	133
IV	187	194	184	196	186	186	130	138	141	155	171	176	144	148
V	151	167	150	164	154	172	180	198	163	175	126	152	151	165
VI	171	174	180	187	189	197	147	158	103	129	152	155	130	133
VII	169	173	136	160	153	169	154	178	157	170	154	167	140	148
VIII	177	180	128	147	185	188	154	170	156	166	125	147	194	216
IX	187	187	184	194	155	156	128	135	67	198	134	158	183	183
X	171	183	181	184	168	176	138	152	116	151	159	164	168	177
Mean	173.0	181.7	163.1	175.5	168.5	175.1	158.5	174.8	146.9	168.6	149.8	161.4	159.5	171.5
±SD	±18.4	±11.8	±24.4	±21.0	±15.0	±14.5	±25.4	±29.3	±30.6	±25.1	±18.2	±11.2	±31.9	±27.8

Haematological values

The mean haematological values of the rats post treatment with the water extract of Herb 25 were as presented in table 2 below. It's of interest to note that all the values obtained were within the normal ranges established for these species of animals (Adamu *et al.*, 2006). Similarly, no significant differences (P>0.05) were observed between the mean values in any of the Herb 25 water extract-treatment group and those in the control group (Table 2).

Table 2: **Mean Haematological Values of Rats Post-treatment with the Water Extract of Herb 25**

Group	PCV (%)	WBC x $10^9/_L$	N(%)	L (%)	M (%)	E (%)	B (%)	B'
A1	39.9 ± 3.1	9.9 ± 2.3	25. 6 ± 4.1	70.2 ± 4.0	2.7 ± 1.6	1.5 ± 0.7	NIL	NIL
A2	41.3 ± 3.4	8.9 ± 1.9	26.1 ± 4.4	70.1 ± 3.8	2.3 ± 1.5	1.3 ± 0.7	NIL	NIL
B1	39.9 ± 3.6	8.5 ± 1.6	25.3 ± 4.5	71.3 ± 5.0	2.2 ± 1.5	1.2 ± 0.8	NIL	NIL
B2	40.9 ± 2.8	8.4 ± 2.1	25.3 ± 4.1	70.5 ± 4.1	2.9 ± 1.7	1.3 ± 0.7	NIL	NIL
C1	42.0 ± 4.4	8.4 ± 1.9	26.3 ± 4.6	69.0 ± 4.7	3.2 ± 2.2	1.5 ± 0.5	NIL	NIL
C2	42.1 ± 4.0	8.7 ± 2.0	26.6 ± 4.8	69.1 ± 5.3	3.1 ± 1.5	1.9 ± 2.2	NIL	NIL
D	40.5 ± 2.8	8.5 ± 2.4	26.7 ± 4.6	69.2 ± 4.3	2.5 ± 1.7	1.6 ± 0.5	NIL	NIL

KEY:

PCV = **Packed Cell Volume**, **WBC = White Blood Cells**, N = **Neutrophil**, L = **Lymphocyte**, M = Monocyte, E = **Eosinophil**, B= **Basophil**, B' = Band cell

Postmortem findings

There were no observable gross lesions in any of the rats in the various experimental groups, A1, B1C1, C1, A2, B2, C2, and D. The microscopic examination of the tissues and organs of all the necropsied rats were normal. Based on the findings from this toxicity study and the LD50 of Herb 25 (1265mg/Kg) Katsayal (2006), it is reasonable to conclude that Herb 25 has a wide margin of safety and it is regarded safe, as an oral preparation for medical use within the experimental doses and periods used in the course of this research work (Ibrahim and Adamu, 2009; 2012).

(c) Histological Studies on the Effects of Chronic Feeding of *Vernonia Amygdalina* Del. Leaves on Rats

Vernonia amygdalina Del. (bitter-leaf tree) is a tropical plant belonging to the family Compositae and is used widely as vegetable and medicinal plant. The study was conducted to evaluate the relative safety of chronic consumption of *V. Amygdalina Del.* in an experimental model (Rat) based on clinical and possible pathological observations. Four groups of male (Wister) rats, aged 4-6 weeks, with average weight of 33.27g were fed formulated feed orally for 65 days. The feed was made up of 25%(w/w), 50%(w/w), and 75%(w/w) air dried grinded leaves of *V. amygdalina* Del., and standard ECWA grower marsh. The rats in the control group were fed on normal feed. The amended feed and water was provided *ad libitum*. All the rats were observed for feed and water intake daily. It was found that the difference in body weight for the treated groups was significantly lower ($P<0.05$) compared to the control group (Table 1).

Table 1: Pre-treatment and post-treatment mean values of water-intake feed and body weight

Parameter/Group		A	B	C	D
Water-intake (ml)	Pre-treatment	14.31 ± 0.56	15.28 ± 0.41	16.62 ± 0.46	14.93 ± 0.62
(Mn ± S.E)	Pre-treatment	14.60 ± 0.35	17.32 ± 2.04	17.01 ± 0.73	16.54 ± 1.98
FEED (g)	Pre-treatment	6.29 ± 0.43	7.02 ± 0.21	7.33 ± 0.26	6.67 ± 0.25
(Mn ± S.E)	Post-treatment	8.69 ± 0.76	9.38 ± 0.84	8.31 ± 0.79	9.41 ± 0.61
Body Weight (g)	Pre-treatment	39.71 ± 1.05	107.94 ± 1.51	143.63 ± 1.64	105.92 ± 1.63
(Mn ± S.E)	Post-treatment	80.17 ± 10.77*	107.34 ± 2.64*	11.35 ± 7.02	148.21 ± 10.16

n = 4
Mn = Mean
S.E = Standard Error
* P < 0.05
** P< 0.01

% V.amygdalina leaves;
A = 25, B = 50
C = 75, D = 0

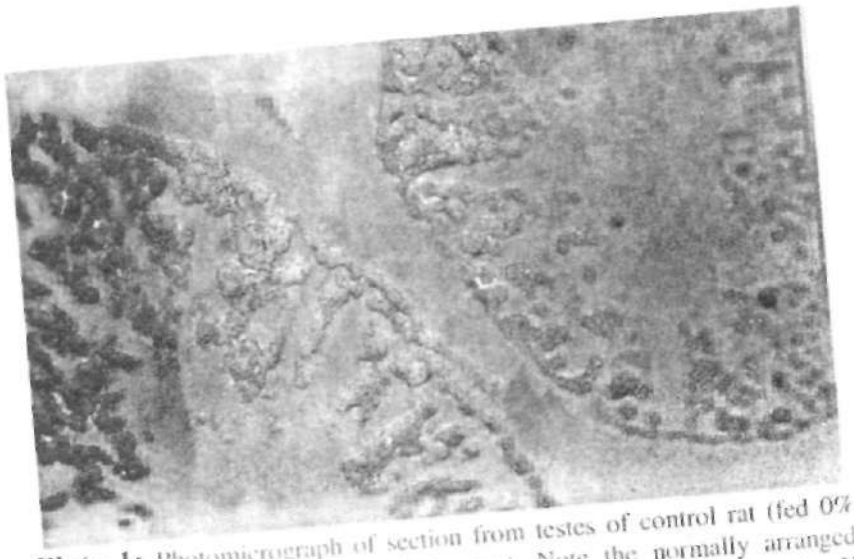


Plate 1: Photomicrograph of section from testes of control rat (fed 0% (w/w) *V. amygdalina* Del. Leaves). Note the normally arranged spermatogenic cells in the seminiferous tubules (H and E stain. X 400).

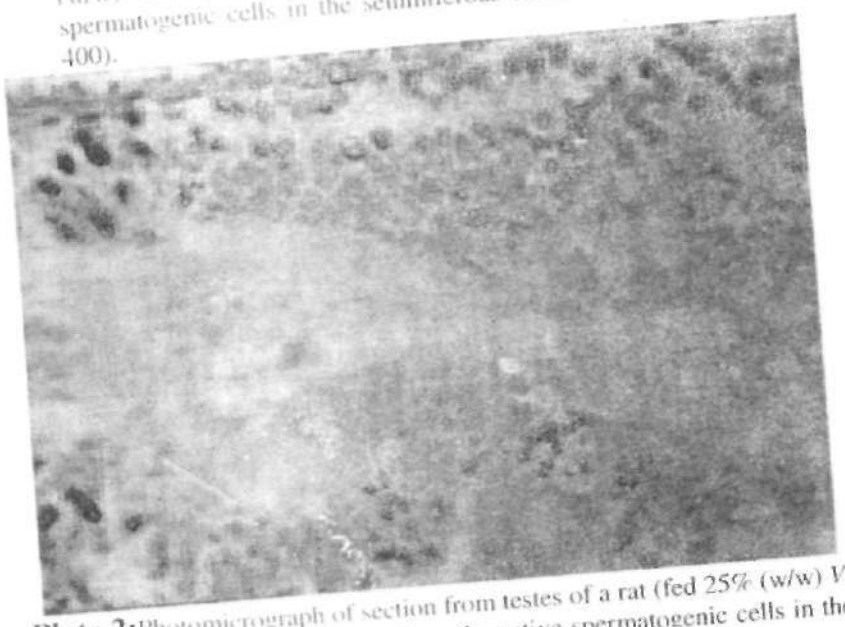


Plate 2: Photomicrograph of section from testes of a rat (fed 25% (w/w) *V. amygdalina* Del. Leaves). Note the active spermatogenic cells in the seminiferous tubules (H and E stain. X 400).

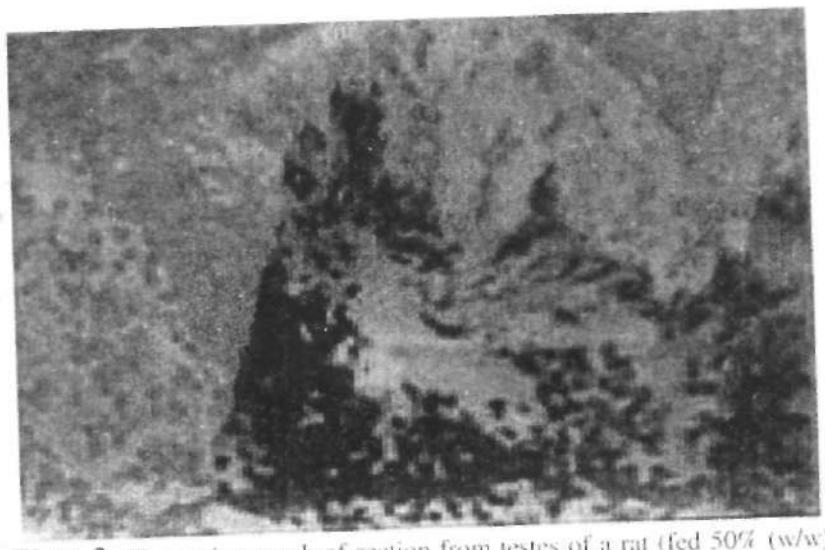


Plate 3: Photomicrograph of section from testes of a rat (fed 50% (w/w) *V. amygdalina* Del. Leaves). Note the activity of the spermatogenic cells in the seminiferous tubules (H and E stain. X 400).

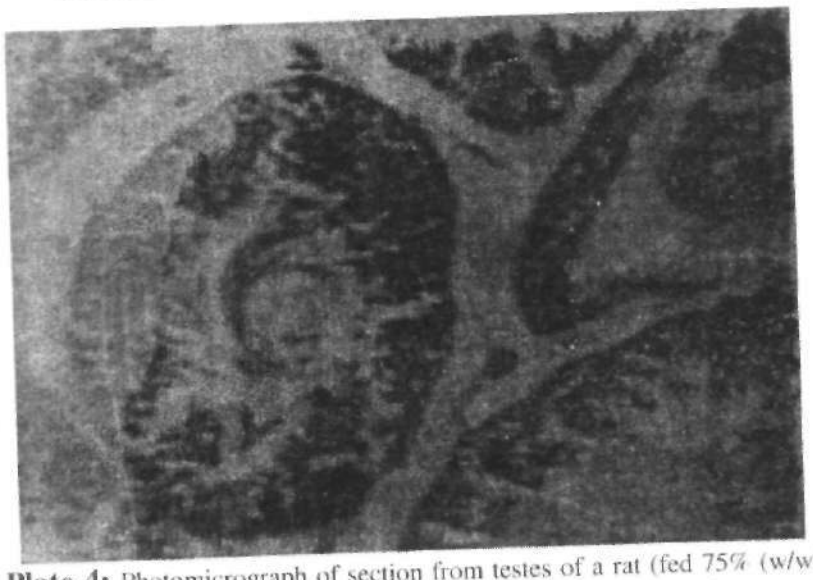


Plate 4: Photomicrograph of section from testes of a rat (fed 75% (w/w) *V. amygdalina* Del. Leaves). Note the abundance and activeness of the spermatogenic cells in the seminiferous tubules (H and E stain. X 250).

After the treatment with *V. amygdalina* Del., the skin of the rats was found to be remarkably light coloured. Microscopically, it was observed that there was active spermatogenesis and erythropoiesis which may indicate possible improvement on the reproductive capacity and amelioration of anaemia when the leaves of the plant are consumed within the experimental doses and periods. The active spermatogenesis may account for the claimed role of the plant in the treatment of infertility (Plates 1, 2, 3 and 4). The cells of the kidneys, livers and testicles had normal morphology and architecture. There was discolouration of the epidermal and dermal cells of the skin. These results and its wide margin of safety with LD50 of 1000 to 2000 mg/kg body weight intraperitoneally in mice indicate that *V. amygdalina* Del. can be considered relatively safe as a medicinal plant and is highly recommended for consumption by both human and animals (Ibrahim *et al.*,1999; 2000).

(d) Elemental Analysis of the Leaves of *Vernonia Amygdalina* Del and it's Biological Evaluation in Rats

Elemental analysis of the leaves of *Vernonia amygdalina* Del. by using an energy dispersive X-ray fluorescence (EDXRF) transmission emission technique at the Centre for Energy Research and Training, Ahmadu Bello University, Zaria (Idris, 1999) revealed adequate concentrations of biologically important elements (Table 1), such as Iron (1500±111ppm) and calcium (10100±895 ppm) that are believed to be essential for normal growth. The plant was screened for toxic or beneficial effects in relation to tissue damage. Sixteen male rats (Wister strains) with average weight of 39.71g and aged 4 to 6 weeks were used. They were fed orally for 65 days with amended feed containing 25 % (w/w), 50% (w/w), and 75 % (w/w) powdered *V. amygdalina* leaves mixed with standard ECWA grower mash. The control rats were fed with standard feed only. At the end of the experimental period, it was found that grossly, the

skin of the treated rats turned lighter in colour than that of the control group. However, microscopical examinations showed that all the various tissues taken including the skin had normal architecture with good cellular morphology.

Table 1: *Concentration of some elements in V. Amygdalina* Del. Leaves

Element	Concentration (ppm ± Standard Error)
K	15400 ± 1450
Ca	10100 ± 895
Mn	87.3 ± 28.7
Fe	1500 ± 111
Cu	23.2 ± LDL
Zn	24.7 ± 5.83
Pb	10.1 ± LDL
Br	33.1 ± 3.54
Rb	35.6 ± 3.39
Sr	76.6 ± 5.95
Th	6.86 ± LDL
Y	15.9 ± LDL
U	6.47 ± LDL
Zr	583 ± 4.60

ppm = Parts per million LDL = Least Detectable Limit
1ppm=1mg/liter of water (NIH,2014a)

On this basis, *V. amygdalina* could be regarded as relatively safe to consume within the experimental periods and at the various doses used. It is also of high nutritive value Ibrahim *et al.* (2002) for both animals and human consumption and could be used to ameliorate anaemia due to its significant amount of iron concentration (15,000 ±111 ppm). It is partinant to note that, the iron concentration found in this study, is within the recommended required range of daily intake of iron for good health by the National Institute of Health (NIH,2014 a); especially being use as an anti-malarial medicinal plant Igile *et al.* (1995) and its effectiveness against *Plasmodium*

falciparum in vitro (Philipson *et al.*,1993).The result of this study also indicates that it could support normal bone formation and healing due to its calcium concentration (10,100 ± 895 ppm) Ibrahim *et al.*, (2001), which is also within the recommended required range of daily intake of calcium for good health (NIH,2014b).

2. Food Security

(a) Screening for *Salmonella* spp. in Some Traditional Food Products in Northern Nigeria

The study was designed to determine the prevalence of Salmonella spp., more specifically *Salmonella typhii* (the aetiologic agent of Typhoid fever) in some traditional foods sold in various markets in Zaria and environs.

Eight traditional food products in northern Nigeria comprising locust bean cake (146), groundnut cake (101), mazarkwaila (7), white candy (52), pankey (10), dakuwa (7), and bean cake (7) all from eight local markets in Zaria and environs were cultured for *Salmonella* spp.

All samples were bought from the following markets, Samaru (108), Sabongari (130), Zaria city (27), Tudun Wada (40), Wusasa (15), Basawa (22), Giwa (136), and Shika (9) based on availability of the food products. At every market a sample of each available product was purchased from each hawker and put into sterile polythene bags. The samples were taken to the laboratory within one hour after purchase. In the laboratory, each sample was aseptically rubbed with sterile swabs and enriched in 6ml of selenite F broth overnight at 37°C. Enriched samples were plated on desoxycholate citrate agar (DCA) and incubated at 37°C overnight. Non-lactose fermenting colonies were thereafter picked and inoculated into triple sugar iron (TSI) agar and urea slants. All isolates that gave K/A +H_2S and urea-negative were further tested using standard methods (Cowan and Steel, 1974).

Despite enrichment in salenite F broth, all the 487 samples were negative for *Salmonella* spp. It's concluded that these traditional local food products do not appear to pose risks of salmonellosis (Typhoid fever) to their consumers (Ibrahim and Adesiyun, 1988).

(b) A Survey of Bovine Tuberculosis at Zango Abattoir, Zaria-Northern Nigeria and its Public Health Significance

Abattoir-based study was undertaken to determine the prevalence of bovine tuberculosis (BTB) in cattle brought for slaughter at Zango-Zaria abattoir. A total of 500 cattle of different breeds, ages, and both sexes were examined at postmortem inspection for evidence of lesions suggestive of tuberculosis. The lesions were found in the lungs, hearts (Plates 1 and 2) and lymph nodes; they were firm, calcified, raised, white to pale yellow tubercles of various sizes that had gritty sound when cut (Calcification).

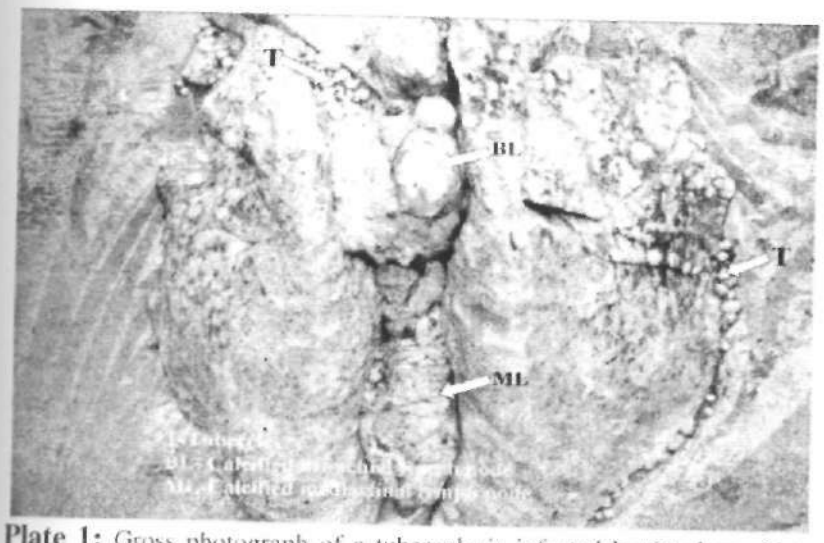


Plate 1: Gross photograph of a tuberculosis infected bovine lung: Note the enlarged calcified (BL-bronchial and ML-mediastinal) lymph nodes and tubercles (T).

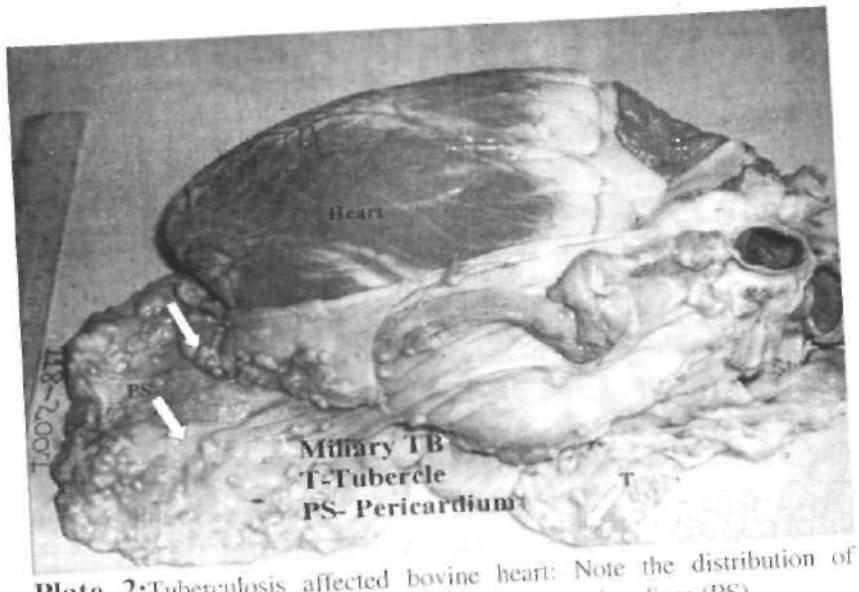


Plate 2: Tuberculosis affected bovine heart: Note the distribution of tubercles (T) on the serous surface of the pericardium (PS)

There was adhesive pleuritis, lung consolidation in some cases and abscesses. Out of the 500 animals examined, 9 (1.8%) suspected lung samples and a heart were sent to microbiology diagnostic laboratory for confirmatory diagnosis. Of these samples, 5 (55.5%) were positive with Ziehl-Neelsen (acid fast) stain representing 1% of the total animals examined. Histopathological findings from the positive samples were caseous necrosis with calcium deposit in the lungs (Plate 3) and hearts.

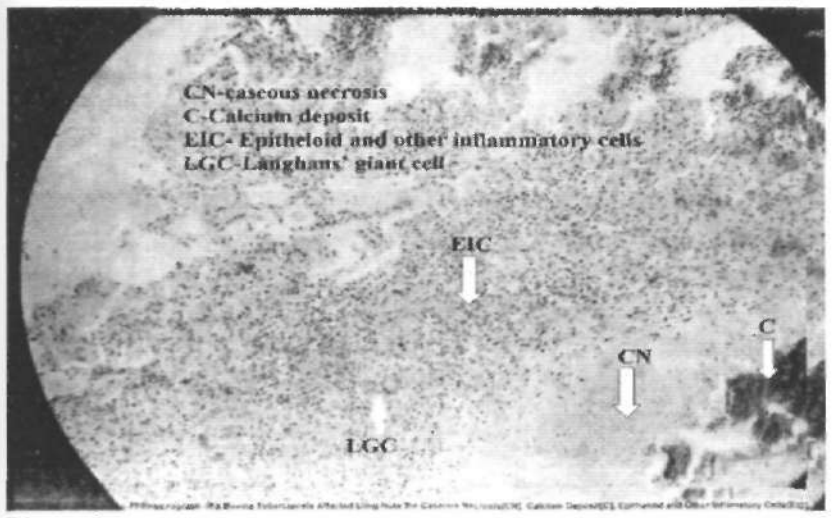


Plate 3: Photomicrograph of the lung section in plate 1: Note the inflammatory cells (EIC), Caseous necrosis (CN) and calcium deposit (C). H and E stain. X 250.

Bovine tuberculosis (BTB) is a chronic bacterial disease of animals and human caused by *Mycobacterium bovis*, and its transmission to human constitutes a public health problem. The 1% prevalence of BTB encountered in this study within 6 months was a small fraction of the true magnitude of the problem in Kaduna State and Nigeria, because the figure was only for recorded cases in one abattoir in Kaduna State out of 36 States of the Federation. This is of public health concern considering the zoonotic nature of the disease. For successful control of BTB in human, the disease must be controlled in cattle and other animals, and that abattoirs play key role in surveillance programmes of BTB (Bello *et al.*, 2009).

(c) Neuronal Degeneration and Meningeal Sialic Acid Complement of Zebu Cattle Experimentally Infected With *Clostridium chauvoei*

For a long time, blackleg was mistakenly assumed to be an exclusively animal disease, because of poor diagnostic tools in

the past. Current reports of the disease in human beings have made research on *C. chauvoei* hot topic. Pathology of the nervous system in blackleg is yet to be exhaustively investigated. In this study, we report for the first time, neuronal degeneration in the brain and the meningeal sialic acid complement of zebu cattle experimentally infected with *C. chauvoei*. Mean meningeal sialic acid complement of the infected cattle was much lower than that of the controls ($P<0.05$), while mean plasma neuraminidase activity of the infected bull-calves that died on day 3 (72 hours) post-infection was five times higher than that of the controls, possibly suggesting that the low sialic acid complement in the infected animals resulted from enhanced cleavage of sialic acid in the meninges by the neuraminidase produced *in vivo* by *C. chauvoei*. This study suggests that sialic acid cleavage from meninges may possibly assist the spread of C. *chauvoei* and opportunistic pyogenic bacterial organisms to the brain to cause meningitis (Useh *et al.*, 2012).

(d) Contagious Bovine Pleuropneumonia (CBPP) at Zango Abattoir, Zaria, Northern Nigeria

Introduction

Contagious bovine pleuropneumonia (CBPP) is a contagious disease affecting the lungs and pleura of cattle and closely related ruminants. It is caused by an infective organism *Mycoplasma mycoides subspecies mycoides* small colonies (MmmSC) bovine biotype (Anonymous, 2006; 2008a).

Contagious bovine pleuropneumonia is one of the reportable diseases according to animal disease control decree 10 of 1988 of the Federal Republic of Nigeria. The disease is listed by the Office International des Epizootes (OIE) as a class A disease making it one of the most economically serious diseases of livestock. The Europian Union (EU) directive 82/894 included

CBPP on the list of diseases that are compulsorily notifiable throughout the EU (Anonymous, 2006). Despite the aforementioned local and international regulations on CBPP, the current prevalence and extent of the disease is largely unknown in Nigeria (Aliu *et al.*, 2000). Therefore, the importance of reporting this and every case of CBPP cannot be overemphasized. There was a decline of CBPP in Africa in the 1970s.This was possible due to 1971 Joint Project 28 (JP28) campaign on eradication of CBPP that started in West African sub region and the annual vaccination programmes against CBPP in many African countries including Nigeria. However, the disease recently has made resurgence and re- emergence during the late 1980s and 1990s in most African countries that had been free of the disease; in some cases, for 25 years or more (Anonymous, 2008b). *Mycoplasma mycoides subspiecies mycoides* was isolated from pneumonic lungs of cattle at slaughter in Maiduguri, Sokoto, and Kaduna abattoirs in Nigeria, as reported by Ajuwape *et al*. (2003).

A case of contagious bovine pleuropneumonia (CBPP) was encountered in a 9-year-old cow during meat inspection at Zango- Zaria, abattoir Kaduna State. Gross examination of the lungs revealed enlargement of the left side of the lung with adhesive pleuritis and massive fibrinous deposition on the pleural surface while the right side of the lung was less affected (Plates 1 and 2). The histopathological findings were fibrinous oedema fluid in the alveoli and other parts of the lung parenchyma as well as necrosis and desquamation of the bronchiolar epithelial cells into the lumen of the bronchioles.



Plate 1: Gross photograph of CBPP affected bovine lung: Note the marbling appearance of the left lung and pleuritis while the right lung was less affected.

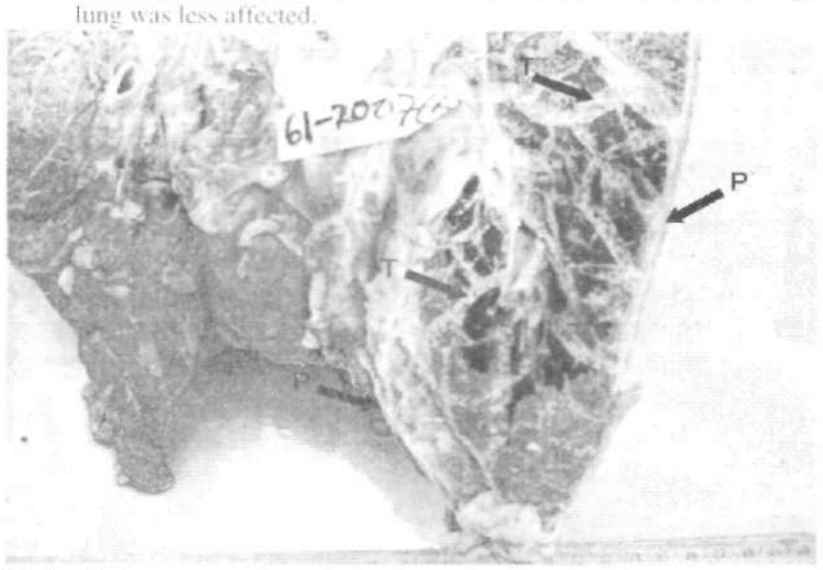


Plate 2: A cross section of the lung in plate 1: Note the thickened interlobular septae (T), marbling appearance and pleuritis (P).

This report is in line with Nigerian and international laws on compulsory reporting of CBPP. It shares experience in field diagnosis of CBPP, and alerts the authorities concern about the continuing existence of the disease among cattle herds and its economic importance in Nigeria. It strongly advocates the need for more strict measures towards effective control and possible eradication of CBPP in the country (Bello and Ibrahim, 2010).

3. Experimental Pathology on:

(a) Pathological Features of Experimental Myocardial Ischaemia-Reperfusion Injury

Introduction

The incidence of heart disease, one of the most important body organs, is well recorded in man. In the United States, ischaemic heart disease accounted for nearly two thirds of the 980,000 annual deaths of cardiovascular origin (Romson *et al.*, 1984). Circulatory disorders account for more than half of the deaths in adults in Europe and disease of the coronary arteries is the foremost cause of death in males, particularly in the 40 to 60 year age group (Leathan, 1977).

Several thorough cardiovascular disease surveys have been conducted in dogs. The incidence of myocardial disease was 3.4 percent in United States (Detweiler *et al.*, 1968), 2.5 percent in Germany (Kersten, 1968) and 1.3 percent (Taylor and Sittnikow, 1968) respectively, and in each study, the incidence increased with advancing age (Ettinger, 1975).

Myocardial infarction is one of the common heart diseases in human. Following infarction, the body attempts to re-introduce oxygenated blood into the infarcted region of the heart, a process known as reperfusion. Reperfusion injury or post-ischaemic injury occurs when molecular oxygen or calcium

ions are re-introduced to the ischaemic or hypoxic tissue by restoration of blood supply to the ischaemic heart (Haglund and Lundgren, 1978). Instead of improving cardiac function, reperfusion results in contracture, cellular necrosis and intracellular calcium overload (Hearse *et al.*, 1973; 1978; Rahamathulla and Ashraf, 1984). This process exacerbates tissue necrosis if the duration of ischaemia is more than 30 minutes, and the extent of injury is proportionate to the duration of ischaemia (Weisfeldt, 1987).

Since the initial observations of phagocytic cells in tissues by Metchnikoff in the late 1800s, a large body of information has accumulated defining the role of phagocytes in acute and chronic inflammatory reactions. When a host tissue is challenged by either immunologic or pathologic agents, an inflammatory reaction occurs, with subsequent clearance of the pathologic agents by phagocytic cells. Tissue injury may result from either the direct effects of the pathologic agent or as a consequence of an inflammatory cell influx, three of the most extensively studied phagocytic cells are the polymorphonuclear leukocytes, the tissue macrophages, and the circulating monocytes. Each of these cells plays a key role in the acute and or chronic inflammatory responses. When pathogenic agents attack host tissues, circulating neutrophils and or monocytes may be recruited from the intravascular compartment to the site of the insult by local generation of soluble mediators. This process of cell migration is thought to involve direct action and chemotaxis (Ward, 1980; Engler *et al.*, 1986).

This may result in irreversible myocardial cell injury by increasing production of oxygen-derived free radicals, superoxide(O^{-}_{2}), hydrogen peroxide (H_2O_2), hydroxyl radical ($OH^{\cdot}$), hypochlorous acid (HOCL) and singlet oxygen (O'_2) by activated granulocytes, and through release of leukocytic lysosomal enzymes into extracellular spaces (Weiss and Lobuglio, 1982; Romson *et al.*, 1983; 1984; Weisfeldt, 1987).

The free radicals are not specific and will destroy structural molecules such as hyaluronic acid and cause peroxidation of lipid membrane, enzyme inactivation and cell death (Hearse *et al.*, 1978; Romson *et. al.*, 1984; Weisfeldt, 1987; Ibrahim, 1988), although these events are important to the repair process, they may also result in the destruction of potentially viable tissue elements (Hess *et al.*, 1985).

Materials and Methods

Forty-two dogs divided into 2 experimental groups were used for experimental myocardial ischaemia-reperfusion injury. In the first group, the hearts of eleven dogs were infarcted by ligating the left descending branch of the circumflex coronary artery for 1.0 hour through a left thoracotomy approach. The ligatures were removed after 1.0 hour to reperfuse the heart for 1 day. Four control dogs had sham thoracotomy incisions. In the second group, the hearts of 16 dogs were infarcted for 1.0 hour as in the first group and the hearts were reperfused for 7 days. Two dogs were used as control and had sham incisions. At the end of the reperfusion periods, the dogs were euthanized and necropsied.

Myeloperoxidase was intended to serve as a biomarker for early detection of Myocardial infarction.

Results

Grossly, the reperfused hearts were rounded and flabby with petechial haemorrhagic areas (Plate 1), while the control hearts were normal. Histopathological studies of the myocardium revealed oedema of some myocytes, necrotic myocardial fibres and leukocytic infiltration (mainly neutrophils with few lymphocytes) in the hearts that were reperfused for 1 day. Sections of the left ventricles of 7-day reperfused hearts showed degenerative changes in the subepicardial zone,

uniform necrosis in the subendocardial zone and necrotic papillary muscle. Leukocytic infiltration consisting of neutrophils, lymphocytes and plasma cells were seen in the sections (Plate 2). There was haemorrhage in the necrotized areas (Plate 3). Evidence of similar histopathological changes were present in the left ventricles of the hearts outside the risk area. In the sections of the hearts reperfused for seven days, there were more necrotic myocardial fibres with loss of nuclei and striations. Macrophages, lymphocytes and plasma cells were seen in 7 days reperfused hearts and not in 1 day reperfused hearts. More leukocytic infiltration in 7 days than in 1 day reperfused heart sections were observed. There was no significant histopathological change in any of the control dogs.

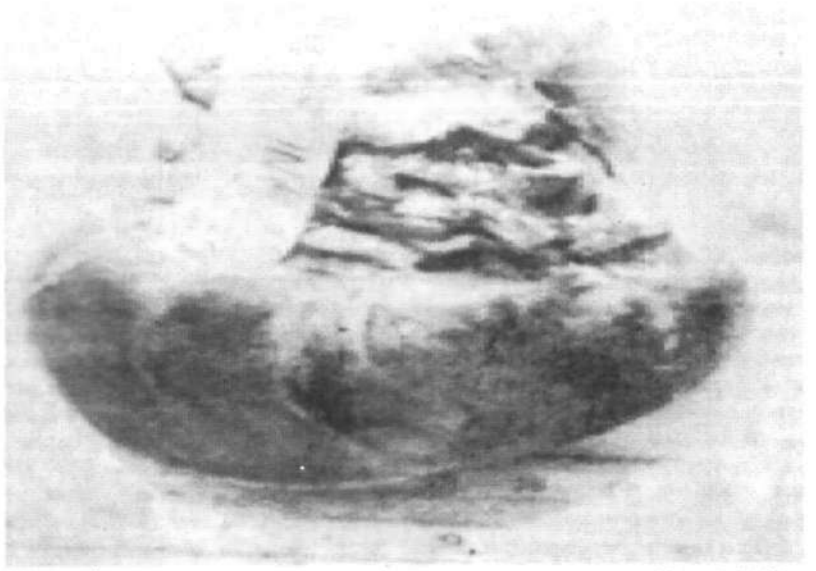


Plate 1: *Gross photograph of reperfused heart showing dilated, flabby and rounded appearance.*

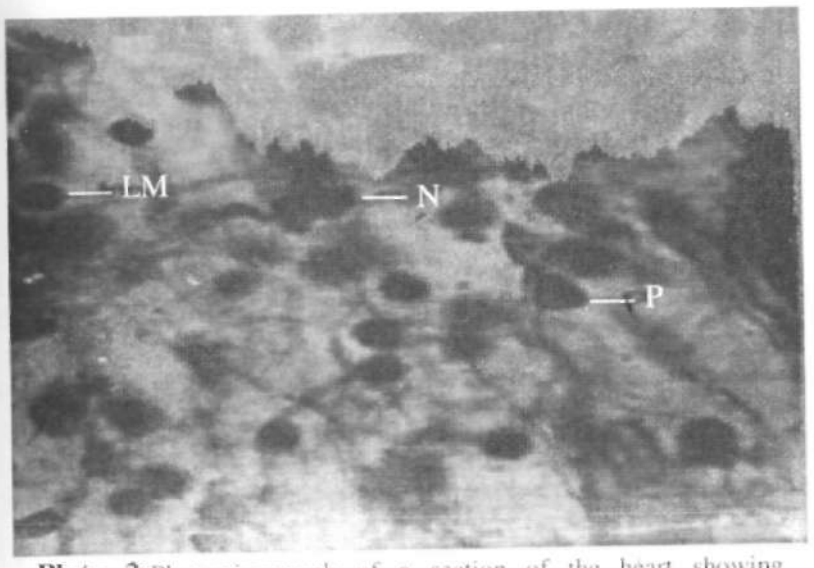


Plate 2: Photomicrograph of a section of the heart showing neutrophils (N), Lymphocyte (LM) and plasma cell (P). H and E stain. X 448.

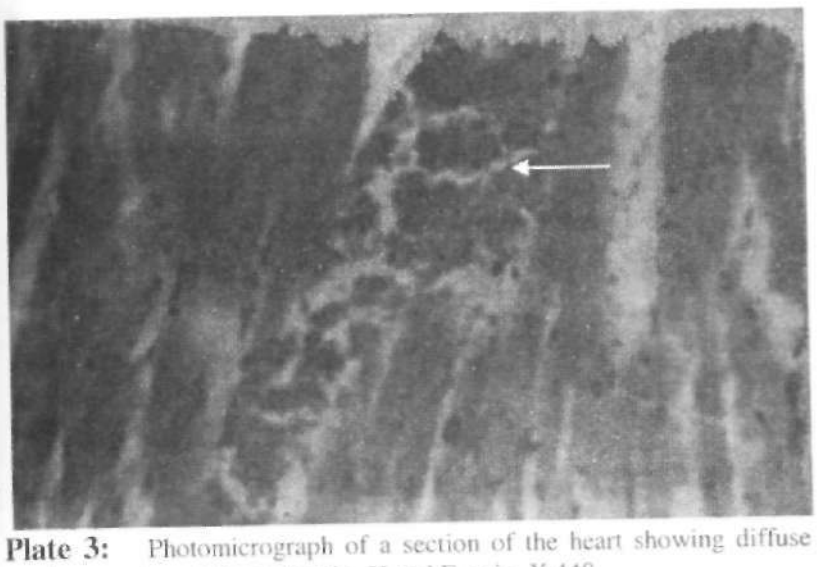


Plate 3: Photomicrograph of a section of the heart showing diffuse haemorrhage (Arrow head). H and E stain. X 448.

This paper reports the pathological features of sequential experimentally induced myocardial reperfusion injury in 1 and 7 days re-perfused hearts in Mongrel dogs (Ibrahim *et al.*, 1990a).

In the course of the study, it was found that Myeloperoxidase was present in white blood cells and other parts of the body, as such, it could not be used as a biomarker. However, it led to the following research on the enzyme.

(b) Neutrophil Myeloperoxidase Deficiency Associated with Canine Hepatozoonosis

Hepatozoonosis is a very important disease in dogs in Nigeria. Hepatozoonosis was reported in Nigeria in 18 dogs. The clinical signs included fever, anorexia, loss of weight, lameness, oculonasal discharge and conjunctivitis. Hematologic findings included leukocytosis due to neutrophilia and eosinophilia. Parasitemia varied from 1 to 9% of the circulating neutrophils in the peripheral blood smears of the dogs examined. *Hepatozoon canis* gametocytes were identified in circulating neutrophils of the dogs. Peripheral blood smears from the dogs confirmed to have natural *H. canis* infection were cytochemically stained for myeloperoxidase. Parasitised neutrophils were myeloperoxidase deficient while non-parasitized neutrophils were myeloperoxidase positive. This is considered important, because deficiency of the enzyme may be responsible for poor response of *H. canis* to chemotherapeutic agents (Ibrahim *et al.*, 1989).

Introduction

Hepatozoon canis is a protozoan parasite, the gametocytes of which inhabit neutrophils of dogs (Craig, *et al.*, 1978; Barton, *et al.*, 1985). The gametocytes may be found in monocytes or neutrophils and stain clear or ice-blue with Giemsa stain (Barton *et al.*, 1985), Dogs are infected with *H. Canis by*

ingesting the brown dog tick, *Rhipicephalus sanguineus* which contains sporozoites. Sporozoites invade the gut wall and undergo schizogony in tissues like cardiac muscle, lymph node, spleen, skeletal and smooth muscle. Rupture of matured schizonts in the muscles results in intense granulomatous reaction. Some merozoites invade neutrophils and monocytes and develop into gametocytes. When *R. sanguineus* ingests blood from an infected dog the gametocytes are released within the gut of the tick. Oocysts containing sporozoites are formed in the tick body cavity (Craig *et al.*, 1978; Barton *et al.*, 1985).

Neutrophils and monocytes from a variety of species contain a heme-enzyme in the primary lysosomal granules known as myeloperoxidase (MPO) (Klebanoff, 1980; Roos, 1980). Sydney *et al.*, (1970) reported myeloperoxidase deficiency associated with defective candidacidal activity in leukocytes with enhanced susceptibility to systemic Candida infection. The deficiency has been described as an autosomal recessive leukocyte disorder. In genetically-determined leukocyte myeloperoxidase deficiency, either the enzyme is completely lacking or it is structurally altered in its enzymatic, heme-binding and antigenic sites. Lippi *et al.*, (1984) studied myeloperoxidase and eosinophil peroxidase activity in healthy subjects and in four totally myeloperoxidase-deficient subjects using cytochemical and biochemical methods. Their studies and others Parry *et al.*, (1981) suggested that myeloperoxidase deficiency has a genetic basis, which would imply a polygenic control of myeloperoxidase formation and activity rather than a single autosomal structural gene inheritance.

Myeloperoxidase deficiency associated with canine hepatozoonosis has not been documented in Nigeria. In this paper, myeloperoxidase deficiency associated with canine hepatozoonosis is reported.

Materials and Methods

This study was conducted on 18 natural cases of *H. canis* infection of dogs in Zaria. Twelve dogs were of the local breed, four were Alsatians and two were cross-bred. The ages of the dogs ranged from 2 months to 6 years. Four of the dogs were of unknown age. There were 14 (78%) males and four (22%) females. All the dogs were confirmed to have hepatozoon by demonstrating *H. canis* gametocytes in thin peripheral blood smears stained with the myeloperoxidase-specific histochemical stain counter-stained with Giemsa stain according to the procedure described by Kaplow (1965). The percentages of parasitized and non-parasitized neutrophils were determined (X100 objective). At the same time, the percentages of myeloperoxidase-deficient and myeloperoxidase-positive neutrophils were determined.

Hematologic values of 13 dogs were determined. Packed cell volume (PCV) was determined by the microhematocrit method and total leukocyte counts (WBC) were estimated using the improved Neubauer hemocytometer. Differential WBC counts were determined as percentages by counting 100 WBCs as described by Schalm *et al.*, (1975). Feces were not examined for ova.

Results

The clinical signs observed in the 18 dogs studied included fever, anorexia, loss of weight, depression, limping, abnormal gait, oculonasal discharge and conjunctivitis.

The hematological findings included leukocytosis due to neutrophilia (16,100-38,400 WBC mm^3). Band neutrophils ranged from 2 to 15% and segmented neutrophils ranged from 58 to 80% of total WBC in seven of the dogs. Out of these seven dogs, two had anemia with regenerative left shift (PCV

for two of the dogs were 18 and 26%, respectively). One of these seven dogs had monocytosis. Eight out of the 18 dogs examined had eosinophilia. Two dogs had a normal hemogram (Table I). The hemogram for five of the dogs was not determined. Parasitemia varied from 1 to 9%. All the parasitized neutrophils were myeloperoxidase negative (Plate 1).

Discussion

The clinical signs and leukocytosis due to neutrophilia observed in this study were similar to reported cases of canine hepatozoonosis (Craig *et al.*, 1978, 1984; Gaunt *et al.*, 1983; Barton *et al.*, 1985; Abdullahi, 1986). Helminths may be responsible for some of the changes in the hematological picture. The level of parasitemia of 1-9% in this study agreed with the report by Craig *et al.*, (1978) and supports the finding that parasitemia is usually at a low level (Barton *et al.*, 1985).

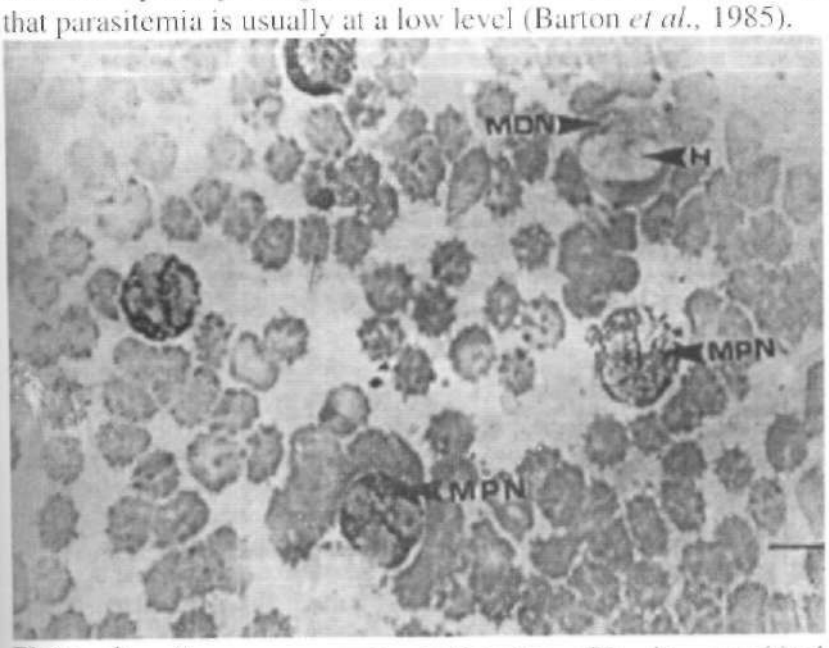


Plate 1: *Hepatozoon canis* gametocyte (H) in parasitized myeloperoxidase-deficient neutrophil (MDN). Non-parasitized myeloperoxidase-positive neutrophils (MPN). Histochemical stain counter-stained with Giemsa stain. Scale bar=10 µm.

Table 1: Hematologic Findings in Dogs Naturally Infected with *Hepatozoon canis*

Case no.	RBC* X 10^6	Hbt (g/100ml)	PCV (%)	WBC (mm^3)	Band Neutrophils(%)	Segmented Neutrophils(%)	Lymphocytes (%)	Monocytes (%)	Eosinophils (%)	Basophils (%)	Total Protein (g/100ml)	Remark
1.	3.6	-	26	28,000	6	72	25	0	0	0	8.3	Slight anemia, leukocytosis due to neutrophilia, regenerative left shift.
2.	6.6	-	38	9500	2	69	19	3	7	0	12.2	Moderate eosinophilia, hyperproteinemia.
3.	5.45	-	36	11,600	1	68	25	0	6	0	8.0	Eosinophilia.
4.	3.6	-	30	9800	5	55	35	0	5	0	7.3	Eosinophilia
5.	7.0	-	31	22,000	5	80	15	0	0	0	10.3	Leukocytosis due to neutrophilia, hyperproteinemia.
6.	6.0	-	36	10,400	3	70	17	1	9	0	4.5	Eosinophilia
7.	6.05	-	32	38,400	6	75	9	0	6	0	7.2	Leukocytosis due to neutrophilia, eosinophilia.
8.	4.8	-	50	24,400	4	75	11	0	10	0	8.5	Leukocytosis due to neutrophilia, eosinophilia.
9.	6.10	-	38	9000	2	46	42	0	10	0	7.3	Eosinophilia.
10.	2.95	6.8	18	31,400	15	63	18	4	-	-	5.5	Leukocytosis due to neutrophilia, hyperproteinemia
11.	4.70	10	30	13,200	1	70	26	1	2	0	8.5	Normal hemogram.
12.	5.10	1.25	38	21,300	2	58	37	1	2	0	7.6	Leukocytosis due to neutrophilia.
13.	6.20	14.4	42	16,100	2	58	22	1	17	0	7.5	Leukocytosis due to neutrophilia, eosinophilia.

The results of this study have shown that male dogs (78%) appeared to be more susceptible to hepatozoonosis than female dogs (22%). This agrees with a previous report by Craig *et al.* (1978). However, Barton *et al.*, (1985) reported 15 cases of hepatozoonosis involving seven male and eight female dogs. The breed of dogs and environmental factors may be responsible for the sex difference in susceptibility. In this country, dogs older than 3 months are more susceptible to hepatozoonosis than dogs of less than 3 months. Similar observations were made by Craig *et al.* (1978), Gaunt *et al.* (1983), Barton *et al.* (1985) and Abdullahi (1986).

In this study, all neutrophils parasitized by *H. canis* were myeloperoxidase deficient. This may be responsible for the inability of the parasitized neutrophils to destroy *H. canis*. It may also be responsible for the poor, or lack of response of *H. canis* to chemotherapeutic agents such as tetracyclines, sulfonamides and chloramphenicol (Craig *et al.*, 1978, 1984; Gaunt *et al.*, 1983; Barton *et al.*, 1985; Abdullahi, 1986), or other common antiprotozoal drugs such as diminazene aceturate (Aliu *et al.*, 1976).

Myeloperoxidase deficiency probably reduces the phagocytic function of neutrophils, and such affected dogs become highly susceptible to systemic infections and recurrent bacterial infection which may account for the fever associated with hepatozoonosis. This is in line with a previous report of myeloperoxidase deficiency associated with defective candidacidal activity of leukocytes resulting in increased systemic *Candida* infection (Sydney *et al.*, 1970).

Hepatozoonosis leads to parasitism of neutrophils by the *H. canis* gametocytes. This leads to myeloperoxidase deficiency in the parasitized neutrophils. With an increase in number of parasitized neutrophils in circulating blood, there will be an increased number of circulating myeloperoxidase-deficient

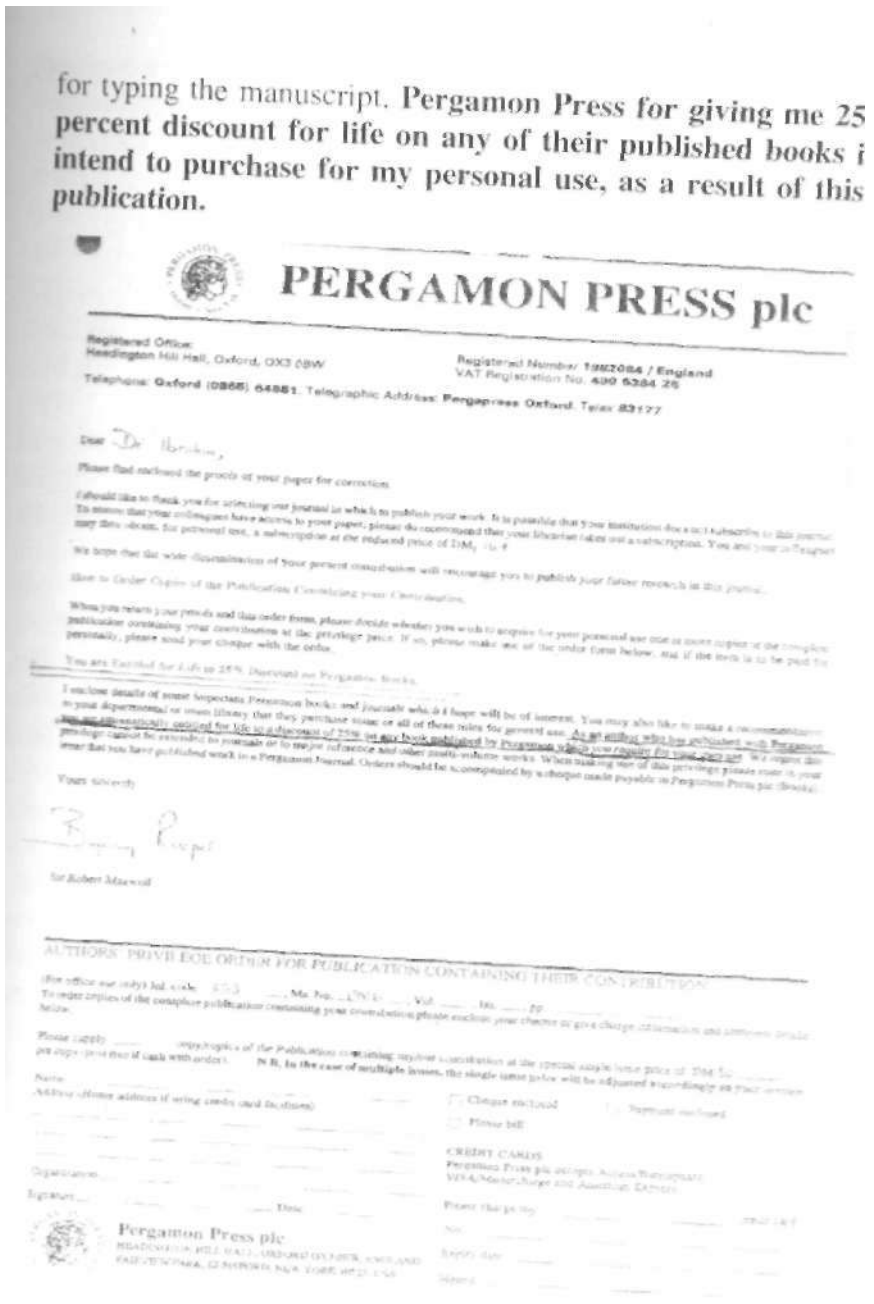
neutrophils. Bendix-Hansen & Nielsen (1985) demonstrated and concluded that an increased number of myeloperoxidase-deficient polymorphonuclear leukocytes in acute leukemia indicates acute myeloid leukemia. The neutrophilic leukocytosis commonly observed in hepatozoonosis (Craig *et al.*, 1978, 1984; Gaunt *et al*, 1983; Barton *et al.*, 1985) may be a compensatory mechanism for the reduced phagocytic function of the parasitized myeloperoxidase-deficient neutrophils.

The ability of *H. canis* to cause myeloperoxidase deficiency may be due to the ability of the gametocytes to inhibit myeloperoxidase biosynthesis in the parasitized neutrophils. It could be possible that *H. canis* utilizes myeloperoxidase in the process of maturation, and also that *H. canis* in the process of maturation, utilizes H_2O_2 (hydrogen peroxide) which is a substrate for myeloperoxidase. The myeloperoxidase in the parasitized neutrophils may be structurally altered. The gametocytes perhaps release some inhibitors of the enzyme. Inhibition of myeloperoxidase leads to inhibition of production of HOCL, (hypochlorous acid) which is toxic (Weiss & Lobuglio, 1982) and this results in decreased phagocytic function of parasitized neutrophils. There is need for more research in the above areas. This paper has reported myeloperoxidase deficiency, and its possible clinical implications, in dogs with *H. canis* infection in Nigeria (Ibrahim *et al.*, 1989).

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(c) Bovine Trypanosomiasis (Trypanosomosis)- *Trypanosoma vivax* Infection

(i) The Effects of Lactose Infusion on the Pathological Manifestations of *Trypanosoma vivax* Infection in Cattle

Except for 4 control calves, 8 calves were experimentally infected using 11 x10^6 *T. vivax* Trypanosomes, intravenously, as a source of neuraminidase. At the peak of parasitaemia, lactose was intravenously infused at the rate of 0.5g kg-1 body weight into 4 of the infected calves, to inhibit the binding of desialylated erythrocytes to Kupffer cells (macrophages), and subsequent erythrophagocytosis. This resulted in amelioration of the anaemia in the 4 infected, lactose-infused calves which showed no gross lesions except for mild anaemia and emaciation (Ibrahim, 1997). In contrast, the other 4 infected, non-lactose infused calves had marked anaemia. The mean PCV values were higher in the infected infused calves while the mean PCV values were lower in the infected uninfused calves (Table 1) with a statistically significant difference ($P<0.05$). The 4 infected, lactose infused calves had mild anaemia and emaciation. The major gross lesions observed in the infected non-lactose infused calves included rough hair coat, emaciated carcases, serous atrophy of coronary fat, enlarged oedematous lymph nodes, and hepato-splenomegaly. Two calves each from the infected lactose infused and non-lactose infused groups and one of the control calves were sacrificed for pathological examinations. In the infected-non-lactose-infused calves there were diffuse hepatic necrosis and erythrophagia by Kupffer cells (Plate 1), dense lymphoid hyperplasia (active lymphoid follicles with plasma cells) (Plate 2) and haemosiderosis. There were haemosiderin laden macrophages in the spleen, liver, kidney, and lymph node. Hypercellular glomeruli, necrosis of the proximal, distal and collecting tubules of the kidney with periglomerular and inter-tubular mononuclear cellular infiltration (Plate 3). There was

minimal myocarditis, while the brain, lung and the intestine of the calves had no significant histopathological lesions. Histopathological lesions in the infected-lactose-infused calves were narrow hepatic sinusoidal spaces (Plate 4), a less dense lymphoid hyperplasia (proliferation of lymphocytes) in the spleen (Plate 5) and lymph nodes and enlarged Bowman's spaces, hypercellular renal glomeruli (Plate 6), compared to the infected-non-lactose infused calves. No significant histopathological changes occurred in the heart, lung, and intestine of the infected-lactose infused calves. No significant microscopic lesions were observed in the liver, kidney, lung, heart, spleen, lymph node, brain, and the intestine of the control calves examined. The results of this study have shown that lactose played a vital role in the prevention or amelioration of tissue injury and cellular reactions in various organs of the body during the course of bovine trypanosomiasis. The finding further supports the safety use of lactose without causing any damage to body organs. As such, its incoporation with other chemotherapeutic agents for treatment of trypanosomiasis is strongly recommended.

Table 1: Profiles of mean PCV for all groups

Days post infection	Infected infused group mean ± Sem	Infected uninfused group mean ± Sem	Uninfected uninfused (control) group mean ± Sem
0	25.5± 1.84	20.0 ±1.67	25.0±1.67
1	25.5± 1.60	20.5 ±1.86	24.5±1.86
2	25.5± 1.62	21.0±1.33	24.5±1.33
3	25.0± 1.54	20.5±1.33	24.5±1.33
4	25.5± 1.52	20.0±1.33	25.0±1.33
5	24.5± 1.73	18.5 ±1.33	25.0±1.33
*6	22.5± 1.60	18.0 ±1.67	25.5±1.66
7	24.0± 1.37	16.5 ±1.86	25.0±1.86
8	20.0± 1.30	13.0 ±1.33	25.0±1.33
9	22.5± 1.65	16.5 ±1.86	25.0±1.86
**10	21.0± 1.42	16.0 ±1.86	25.0±1.86
11	18.5± 1.06	16.5 ±1.46	26.0±1.46
12	19.5± 1.06	20.0 ±1.46	26.0±1.46
13	18.0 ± 1.06	17.0 ±1.46	25.0± 1.33

*the day when lactose infusion started

**the last day of lactose infusion

SEM: ±The standard error of the means.

The difference between the mean PCV values of the infected infused calves and the mean PCV values of the infected uninfused calves was statistically significant ($P < 0.05$).

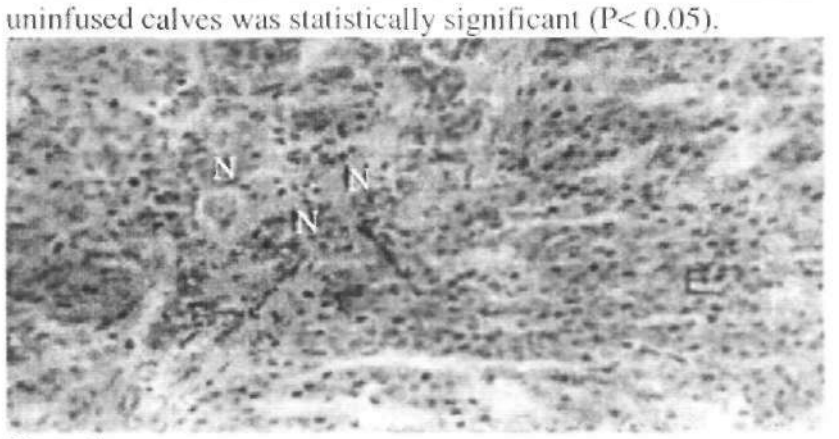


Plate 1: Photomicrograph of section from the liver of a *T. vivax* infected calf.

Note necrosis of the hepatocytes (N), erythrophagia at the portal area and dilated sinusoids H and E stain. X 400.

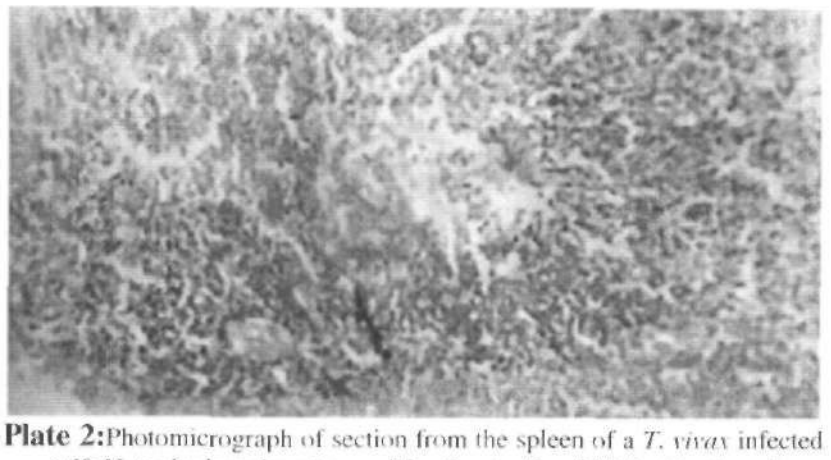


Plate 2: Photomicrograph of section from the spleen of a *T. vivax* infected calf: Note the lymphocytes proliferation (active follicles) in the spleen. H and E stain. X 400.

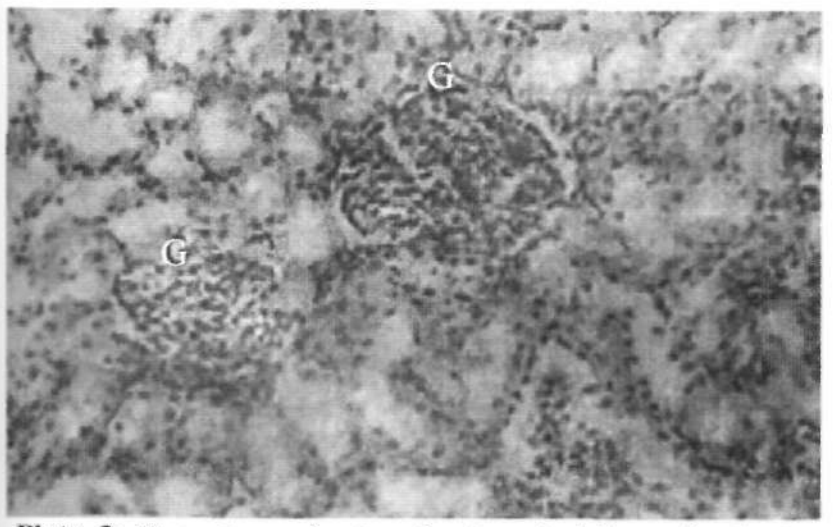


Plate 3: Photomicrograph of section from the kidney of a *T. vivax* infected calf.

Note hypercelluler glomeruli, adhesion of the parietal layer of the glomeruli to the Bowman's capsule (G), necrosis of the renal tubules and periglomerular and inter-tubular mononuclear cellular infiltration. H and E stain. X 400.

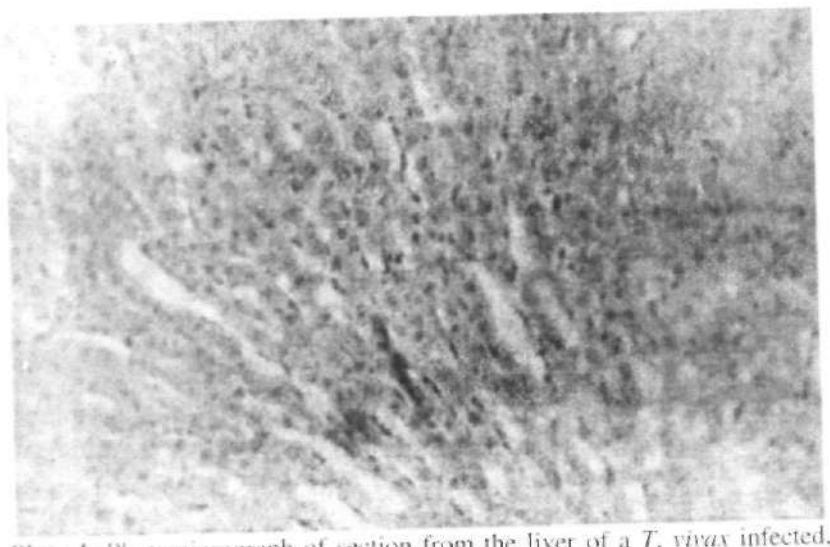


Plate 4: Photomicrograph of section from the liver of a *T. vivax* infected, lactose infused calf. Note fatty degeneration in the hepatocytes and scanty erythrophagia at the centrilobular area. H and E stain. X 400.

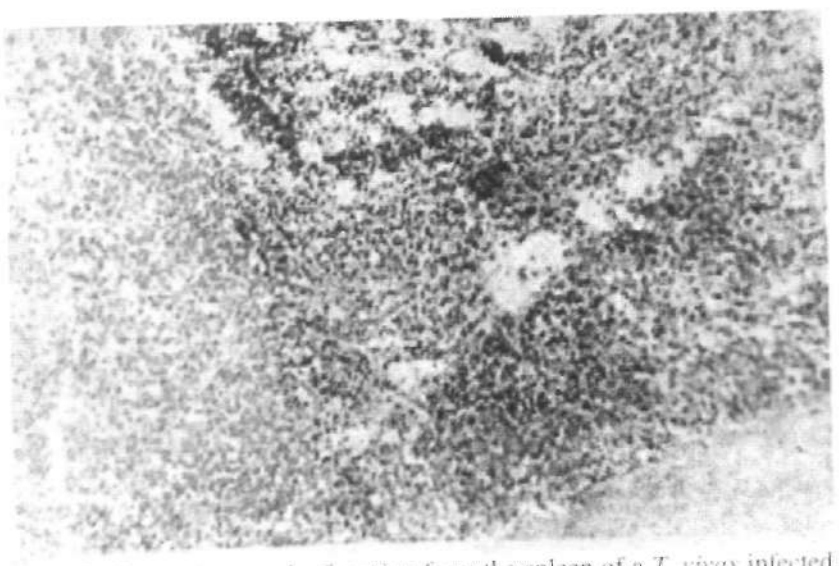


Plate 5: Photomicrograph of section from the spleen of a *T. vivax* infected, lactose infused calf. Note the less dense lymphoid hyperplasia in the spleen H and E stain. X 400.

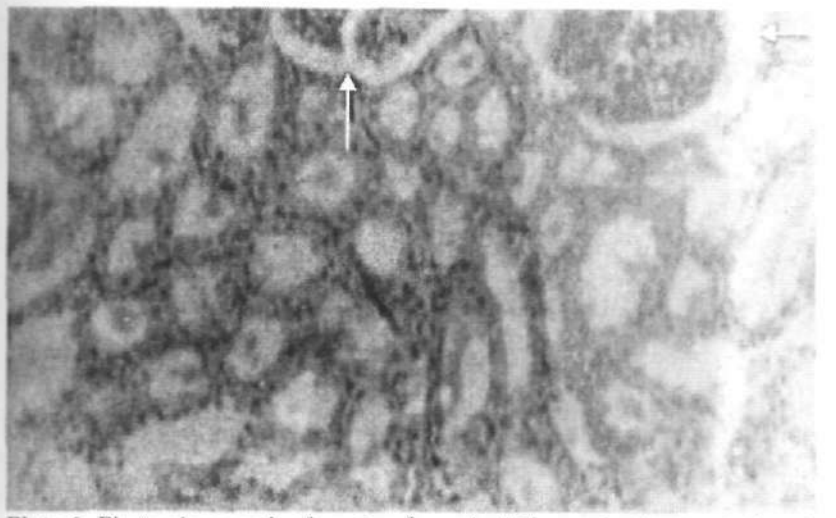


Plate 6: Photomicrograph of section from the kidney of a *T. vivax* infected, lactose infused calf: Note the wide Bowman's space with no adhesion of the glomeruli to the Bowman's capsule (Arrow heads). Also note the absence of renal tubular necrosis. H and E stain. X 400.

It can be concluded that, the inhibitory effect of lactose on tissue and cellular injury and erythrophagocytosis by Kupffer cells as shown in the infected non-lactose infused calves (Plates 1, 2 and 3) and that of infected lactose infused calves (Plates 4, 5 and 6) can result in the following: Amelioration of anaemia (Table 1), prevention of destruction of the normal morphology of the tissues and cells of the liver, spleen and kidney to near normal in trypanosomiasis. This implies that probably the normal physiological functions of these organs, such as detoxification of toxic substances by the liver, excretion of such toxic substances by the kidney of the animals suffering from trypanosomiasis may be restored by concurrent lactose infusion during chemotherapy of trypanosomiasis or by using lactose as a base for trypanocidal agents (Ibrahim *et al.*, 2005).

(ii) The Role of Kupffer Cell Surface Lectin in Erythrophagocytosis following Acute Ruminant *Trypanosoma vivax* Infection

Introduction

Reduction of erythrocytes life-span and erythrophagocytosis are consistent contributory factors in the aetiology of the anaemia of cattle trypanosomiasis (Mackenzie and Cruickshank, 1973; Valli *et al.*, 1978; Valli *and* Forsberg, 1979; Dargie *et al.*, 1979a,b). Significant cleavage of erythrocytes surface sialic acids during peak trypanosome parasitaemias and thence highest production of trypanosome sialidase (Esievo; 1979) were incriminated in the shortening of erythrocytes life-span and erythrophagocytosis of bovine trypanosomomiasis (Esievo *et al.*, 1982). Much support for this was found in the numerous quantitative and qualitative biochemical differences between the erythrocytes sialic acids of the trypanosusceptible Zebu cattle and the trypanotolerant Ndama cattle, which also produced favourable explanation for latter's trypanotolerance (Esievo *et al.*,1986; Esievo *et al.*,1990; Shugaba *et al.*, 1994).

Earlier, it was postulated that at peak parasitaemia, trypanosome sialidase cleaves off erythrocytes surface sialic acids significantly causing physico-chemical damages on the desialylated erythrocytes rendering them more prone to premature phagocytosis and destruction by the reticuloendothelial system (Esievo *et al.*, 1982). We have very recently shown that these physico-chemical changes are in fact galactose residues exposed on the membranes of erythrocytes in the *T. vivax* infected cattle, confirmed by the binding and rosetting of such desialylated erythrocytes, by using freshly isolated rat peritoneal macrophages (Omage; 1996, personal communications). Similar erythrocytes galactose exposure was achieved with bacterial commercially-produced sialidase

(Jancik *et al.*, 1978; Kolb and Kolb-Bachofen, 1978; Muller *et al.*, 1981; Muller and Schauer, 1983; Muller *et al.*, 1983a; 1983b).

Materials and Methods

Experimental Animals

Four (4) young adult goats of about 12 months old and 4 cattle averaging about 18 months old were used for this project. These animals were part of another experiment where they were experimentally infected with T *vivax* by jugular venipuncture. All the experimental animals were screened for any detectable systemic infections, dewormed with albendazole and tick bathed with chlorfenvinphos.

In vitro studies were performed with goats using lactose solution at 10mg per ml. whereas *in vivo* studies were performed with the infected cattle, 2 of which were infused with lactose at 0.50g per kg of body weight, three times a day at 4 hours intervals for 4 days, while the other two remained uninfused. All experimental animals were sacrificed immediately after their first peaks of parasitemias.

Samples Collection and Preparation:

i. Ten (10) ml of blood was collected by jugular venipuncture from each of the infected and non-infected goats and from the infected; lactose-infused and infected non-infused cattle. The blood samples were transferred into vacutainers with ethylene diamine tetra-acetic acid (EDTA) as anticoagulant. The blood was mixed with phosphate buffer pH 7 and centrifuged at 2500 revolutions per minute (rpm) at 2°C for 5 minutes (Beckman Model J-21B). The supernatant was discarded and the sedimented erythrocytes were

resuspended in phsophate buffer and re-centrifuged. The centrifugation step was repeated until the cell pellets were clear. The packed cell volume (PCV) and haemoglobin concentration (Hb) for all the animals (goats and cattle), infected, infected-lactose infused and the control were determined using haematocrit method.

ii. One gram of the liver from each goat and cattle was used. The liver samples were perfused with 10ml of warm isotonic 0.9% sodium chloride (NaCl) solution and 5ml of 0.2% (0.4g/200ml) pronase E (Sigma) in Hank's balance salt solution. The liver was excised, cut into small pieces in the petri dishes and incubated at 37°C in 15ml of pronase E solution with continuous gentle shaking for 20 minutes. The cell suspension was filtered into plastic test tubes through nylon gauze. The filtrate was centrifuged at 2500rpm at 2°C for 5 minutes (BECKMAN Model J-21 B centrifuge). The supernatant was discarded; the cell pellets (Kupffer cells) were gently resuspended in cold Hank's balanced salt solution.

The resuspended cell pellets were recentrifuged at 2500rpm at 2°C for 5 minutes. The centrifugation step was repeated until the cell pellets were clear. The cell pellets (Kupffer cells) were finally resuspended in *Medium*199E (Sigma) mixed with 10% (0.3g/3ml) Bovine calf serum (Randox) in plastic test tubes in the presence or absence of 10.8mg/ml Lactose in case of goats, for *in vitro* study. Lactose infusion replaced this latter step in the case of cattle. The cells were incubated at 37°C for 18 hours in humidified air containing 5% $C0_2$ in the presence of desialylatederythrocytes (from *T. vivax* infected goats and cattle) or normal erythrocytes of uninfected goats and cattle respectively, for erythrocyte macrophage (Kupffer cell) interaction. After the erythrocyte-macrophage interaction, smears of the cells were made on clean glass slides. The smears were allowed to dry at room temperature, fixed in methanol for

5 minutes and stained with Giemsa stain for 30 minutes. The slides were allowed to dry and were examined under the Zeiss microscope (X 40 objective).

For quantification, the suggested method by Schauer *et al.*, (1984) was used. One hundred Kupffer cells (macrophages) were counted and percentage macrophages with binding and erythrophagocytosis, percentage macrophages with phagocytosis but without binding, percentage binding (rosette) and percentage macrophages that have neither binding nor erythrophagocytosis (Plates 1, 2 and Tables 1 and 2) were determined and the data were recorded.

It appears that homologous macrophages, particularly liver Kupffer cells in trypanosome infected animals may operate an accelerated rosetting of desialylated erythrocytes for onward erythrophagocytosis in *T. vivax* infected ruminants. This process partly involves the liver Kupffer cells surface lectin, binding onto the galactose exposed on the red cell membranes after desialylation.

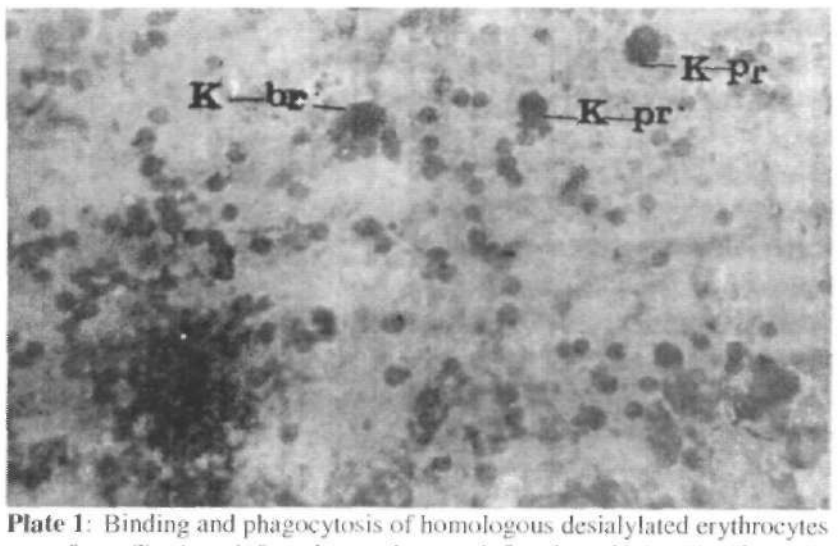


Plate 1: Binding and phagocytosis of homologous desialylated erythrocytes from *T. vivax* infected, non lactose infused cattle by Kupffer cells isolated from liver of the same cattle. K-br (Kupffer cell with bound erythrocytes); K-pr (Phagocytosed erythrocytes). Giemsa stain. X 400.

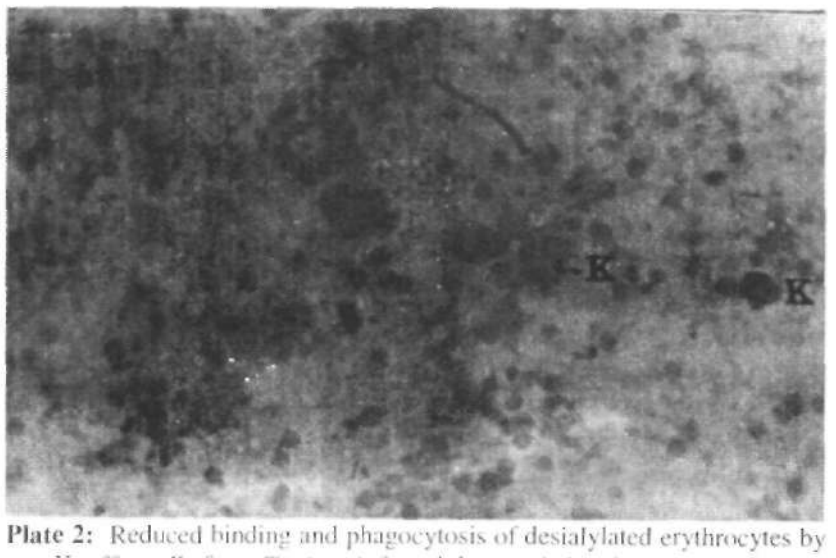


Plate 2: Reduced binding and phagocytosis of desialylated erythrocytes by Kupffer cells from *T. vivax* infected, lactose infused cattle (K). Giemsa stain. X 400.

Table 1: Binding and phagocytosis of normal and desialylated red cells from caprine *T. vivax* infection in the absence or presence of lactose (unit)

Case No.	Number of phagcytized RBC/*KPC	Treatment	Binding and phag-cytosis (%)	Phag-cytosis only (%)	Binding only	No binding No phagocytosis
1(a)	1-2	Desialylated RBC +Kupffer cells +1.0ml bovine calf serum.	19	12	40	29
1(b)	0-1	Normal RBC +Kupffer cells +1.0ml bovine calf serum.	1	2	29	68
1(c)	0-1	Desialylated RBC +Kupffer cells +5.0ml lactose +1.0ml bovine calf serum.	2	2	8	88
1(d)	0-1	Normal RBC +Kupffer cells +5.0ml lactose +1.0ml bovine calf serum.	0	1	2	97
2(a)	1-2	Desialylated RBC +Kupffer cells +1.0ml bovine calf serum.	12	7	18	63
2(b)	0-1	Normal RBC +Kupffer cells +1.0ml bovine calf serum.	3	2	10	85
2(c)	0-1	Desialylated RBC +Kupffer cells +5.0ml lactose +1.0ml bovine calf serum.	2	4	4	90
2(d)	0-1	Normal RBC +Kupffer cells +5.0ml lactose +1.0ml bovine calf serum	0	0	3	97

* KPC - Kupffer Cell

Table 2: Binding and phagocytosis of normal and desialylated red cells from bovine *T.vivax* infection in the absence or presence of lactose (unit)

Case no.	No. of RBC/*KPC	Treatment	Binding and Phagocytosis (%)	phagocytosis only(%)	Binding only (%)	No binding No phagocytosis (%)
1	RBC 1- 2	Desialylated RBC +Kupffer cells + 1.0ml bovine calf serum	13	4	45	38
2	RBC 1-3	Desialylated RBC +Kupffer cells + 1.0ml bovine calf serum.	14	9	36	41
3	RBC 0-1	Desialylated RBC +Kupffer cells +infused lactose + 1.0ml bovine calf serum.	1	1	34	64
4	RBC 0-1	Desialylated RBC +Kupffer cells +infused lactose + 1.0ml bovine calf serum.	0	0	10	90
5	RBC 0-1	Normal RBC+Kupffer cells +1.0 ml bovine calf serum.	0	0	5	95

* KPC - Kupffer Cell

Summary

In the present study, binding and subsequent erythrophagocytosis was inhibited in ruminants (goats and cattle) by lactose, both *in vitro* and *in vivo*. This agreed with earlier experimental reports in rats by Kolb and Kolb-Bachofen (1978) and Muller *et al.*, (1981). The authors reported that adherence (binding) was mediated by β-glycosidically bound galactose on desialylated erythrocytes to the macrophage (Kupffer cell) surface lectin. Both the binding and erythrophagocytosis were inhibited in rats by lactose (Kolb and Kolb-Bachofen, 1978; Muller *et al.*, 1981; Kuster and Schauer, 1981) and in ruminants in the present study. The most important beneficial inhibitory effect of lactose on binding and subsequent erythrophagocytosis, was the amelioration of the anaemia in ruminant trypanosomiasis (Ibrahim, 1997).

4. Diagnostic Pathology on Routine Diagnostic Services to Our Immediate Community and Referred Cases

(a) Multiple Congenital Malformations Associated With Incomplete Twinning in Yankassa Sheep

Case History

An ewe was presented at the Large Animal Clinic of Ahmadu Bello University, Zaria with a set of abnormally joined twins on 28th January, 1986, shortly after she had lambed. The ewe was physically normal and had lambed two times previously both resulting in normal lambs.

The two lambs were joined at the abdomen. The smaller lamb was anencephalic and had a proboscis in place of nostrils. Both lambs could move and respond to pin prick. The lambs died shortly after an attempt was made to force one to suckle. Lateral radiographs of the twins were taken and the animals submitted for necropsy examination.

Radiographic examination revealed that the lambs were joined at the abdomen and had a common liver. In one of the lambs " A" the skeletal system appeared well developed, except for some deviation of the vertebral column involving the lumbar vertebrae. The other lamb " B" had anencephaly but the rest of the skeletal system appeared fully developed (Plate 1).

Necropsy examination confirmed that the diplopagus twins were joined at the abdomen and had a common umbilicus. The bigger lamb "A" (Plate 1) had a well developed head and scoliolordosis. The right leg had aplasia of muscles of the femur. The posterior third of the tail was kinky and the left cornea was more opaque than the right one. The lung floated in water indicating that it had breathed before death.

The smaller lamb (B) had anencephaly with a proboscis in place of nostrils; but ears were well formed. Its lung sank in water indicating it had not breathed. It also had scabbard trachea. The twins had common liver, kidney, and umbilicus. Histopathology of the lung sections of the smaller lamb showed evidence of pulmonary oedema, and melanosis.

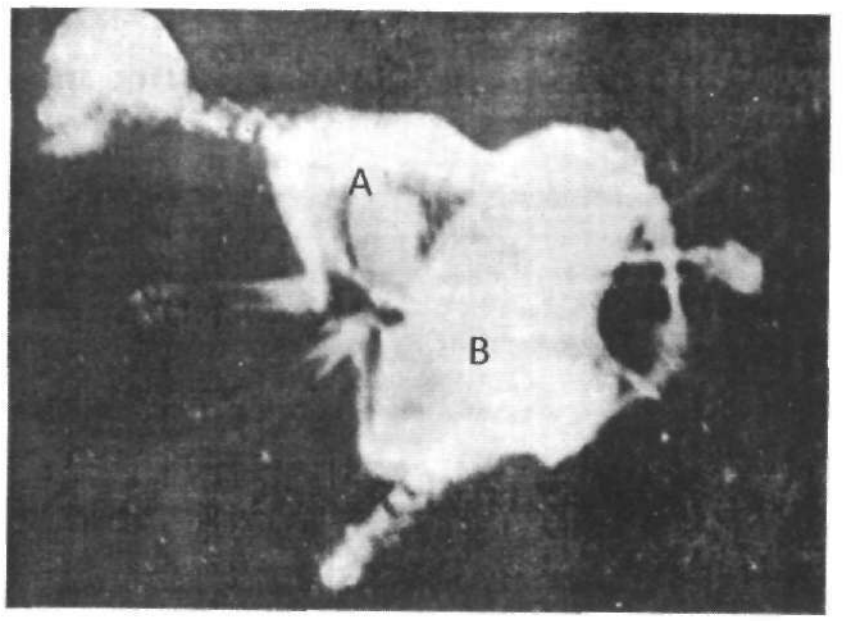


Plate 1: Multiple congenital malformations associated with incomplete twinning in Yankassa Lambs: Note the bigger lamb (A) had a well developed head and the smaller lamb (B) had acrania and was anencephalic.

Discussion

There are many reports of congenital malformations associated with Siamese twins in sheep (Dennis, 1975). Asymmetry has however been reported in co-joined lambs (Arthur, 1956; Potter, 1961; Willis, 1962). Gordon and Lowe (1973) reported double monster and poor muscular development in calves joined at the ventral midline from the brisket to the umbilicus with one of the calves more completely-formed than the other. Anencephaly is rare in sheep (Dennis and Leipold, 1972), but has been reported in association with scoliosis and other malformations in a Yankassa lamb (Gyang *et al.*, 1984). Common liver has also been observed in co-joined calves

(Gordon and Lowe, 1973). Proboscis-like nose was also reported associated with cyclopia in a puppy (Njoku *et al.*, 1978). The malformations observed in the case reported here were too severe and not compatible with life (Ibrahim *et al.*, 1987).

(b) Monocephalus Dipygus Twins in Sokoto Red Goat

Case History

Dipygus female kids were presented at the Faculty of Veterinary Medicine, Ahmadu Bello University, Zaria on 29th August, 1988 for necropsy.

The nanny goat delivered triplets on 28th August, 1988 in a normal way. The three kids were females and one was normal while the other two kids were dipygus twins. Kid A of the dipygus twins was delivered alive and died in the night of the day when it was delivered. Kid B was not delivered alive (Plate 1).

The nanny goat had three previous normal deliveries. There was no previous history of congenital malformation in the flock.

Physical Examination

The two kids were joined at the scapular region. Kid B was joined to kid A at the left scapular region (LS). They had monocephalus, two well- developed separate ears (polytia), tetrabrachius, tetrapus, separate spinal columns. Each twin had well developed vulva and tail (Plate 1).

Necropsy Examination

Necropsy examination revealed that the dipygus kids had a common heart, rudimentary lungs and single gastrointestinal tract. The thoracic aorta of each kid was joined at the base of the common heart.

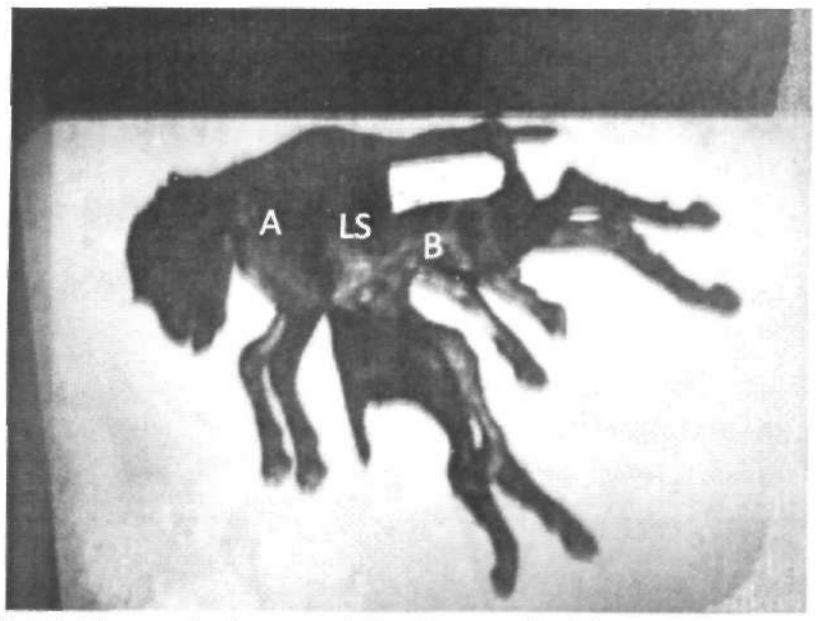


Plate 1: Photograph of monocephalus dipygus twins joined at left scapular region (LS). It had polytia (two ears), tetrabracius, tetrapus, two tails, and separate spinal columns.

Discussion

The study of congenital malformation throws light on developmental processes (Dennis and Leipold, 1972). Literature on dipygus twins is rare especially in goats. Similar malformations observed in this report were reported in sheep previously by Dennis (1975). Cyclopia associated with dipygus twins as reported by Dennis (1975) in sheep was not observed in the present report. This paper reports too severe

malformations that are not compatible with life. Reports on the malformations reported here are very rare (Ibrahim, *et al.*, 1991).

(c) Multiple Congenital Defects in a Bunaji Bull

Case History

The congenitally malformed two-and-half year-old Bunaji bull was purchased by a butcher from a local market at Anchau, Kaduna State, northern Nigeria for slaughter at Zaria abattoir. The bull had gone through many hands and the original owner could not be traced. It was however, gathered that the dam of the malformed bull was disposed off long ago after calving due to superstitious belief.

On arrival of the bull at the Faculty of Veterinary Medicine, Ahmadu Bello University, Zaria, a detailed physical examination was carried out. Blood samples were collected for haematological and parasitological examinations, and serum chemistry analysis was done using the method described by Margi (1995). The bull was purchased to be used for postgraduate research. Unfortunately few weeks after its arrival at the Faculty of Veterinary Medicine, Ahmadu Bello University, Zaria, it died. A detailed postmortem examination was carried out on the carcass.

Result

On physical examination, the bull had notomelia characterized by presence of two additional hypoplastic hindlimbs attached to the right lumbosacral region. There were fully developed normal fore and hind limbs (two in each case). The bull was a monorchid with a hypoplastic scrotal sac devoid of palpable testicle located at a point between the extra hindlimbs and a second fully developed scrotum that contained the fully

developed testicle and it had two prepuces, which were adjacent to each other, and each had a patent preputial orifice (Plates 1 and 2).

The bull had a fully developed penis that was protruding and retracting through the left prepucial orifice, while the second penis on the right side was hypoplastic and palpable in the right prepuce. There was a well-developed but deviated tail that extended from the sacral bone at the point of the attachment of the extra hindlimbs to the level of the Hoch joint. It also had a well-developed patent anus at the posterior lateral region of the right hind-limb. An extra rudimentary atresic anus was found between the extra hind-limbs. Each of the two additional hind-limbs had femur, tibia, patella, tarsal, and metatarsal bones, hooves and the associated joint of the hind limbs (Plates 1, 2, and 3).

Postmortem examination revealed that the bull had kyphosis from the 1st to the 9th thoracic vertebrae and scoliosis that involved the lumber vertebrae. The tuber coxae of the bull were asymmetric; the right tuber coxae measured 3.5cm by 4.0cm, while the left one measured 2.8cm by 7.5cm. The extra hind limbs were attached to the lumbosacral region by hypoplastic pelvic bones and muscles. The shafts of the hypoplastic ilia, tuber coxae and the heads of the femurs of the two additional hind limbs were attached to the right ilium and tuber coxae. The left extra hind limb was attached to the right hind limb at the ischium and the tuber ischii of the two extra hind limbs caudal to the hypoplastic obturator foramen through which the sciatic nerve that supplied the additional limbs passed. The muscles of the additional hind limbs were hypoplastic. The origin of the extra hypoplastic penis was at the point where the two extra hind limbs joined. There were two recta; one was fully developed as a continuation of the gastrointestinal tract and led directly to the patent anus. The second rectum was hypoplastic and located between the

aponeurosis of the abdominal muscles and the atresic anus that was between the extra hind limbs (Plate 3). The bull also had a single hypertrophied kidney that measured 7cm by 16 cm, there was an intrapelvic urinary bladder that was attached to the pelvic cavity, dorsally. The inlet to the pelvic cavity measured 7cm by 10 cm. Other postmortem findings were subcutaneous oedema, hydrothorax, congested lungs, hypertrophied heart and traumatic reticulitis due to ingestion of a sewing machine needle, which was finally diagnosed to be the cause of the death of the bull.

Results of serum chemistry and haematological analyses were as presented in tables 1 and 2, respectively. The result of the serum chemistry analyses indicated normal values of the total plasma protein (52.0g/L), alkaline phosphatase (37.0 IU/L), alanine (7.1 UI/L) and aspartate aminotransferase (29.0 UI/L).The values of blood urea nitrogen and creatinine (11.8 mmol/L and 309.4 mmol/L respectively) were high (Table 1). Most haematological values were normal except for moderate leukocytosis (Table 2) just before the bull died.

Table 1: Serum chemistry tests results on the 1st and 2nd days of sampling

Parameter	Day 1	Day2
Total protein (g/L)	68.0	52.0
Alkaline phosphatase (I U/L)	32.0	37.0
ALT (I U/L)	6.8	7.1
BUN (mmol/L)	11.1	11.8
Creatinine (mmol/L)	318.2	309.4
Sodium (mmol/L)	126.0	118.0
Potassium (mmol/L)	5.4	5.2
Chloride (mmol/L)	108.0	122.0
AST (I U/L)	26.0	29.0

Table 2: Result of haematological analyses on the 1st and 2nd days of sampling

Parameter	Day 1	Day 2
PCV (%)	22.0	15.0
WBC ($x10^9$/L)	9.3	14.0
RBC ($x10^{12}$/L)	3.8	3.1
Neutrophils (%)	37.0	41.0
Lymphocytes (%)	54.0	58.0
Monocytes (%)	3.0	1.0
Eosinophils (%)	5.0	1.0
Basophils (%)	0.0	0.0
Band (%)	1.0	0.0

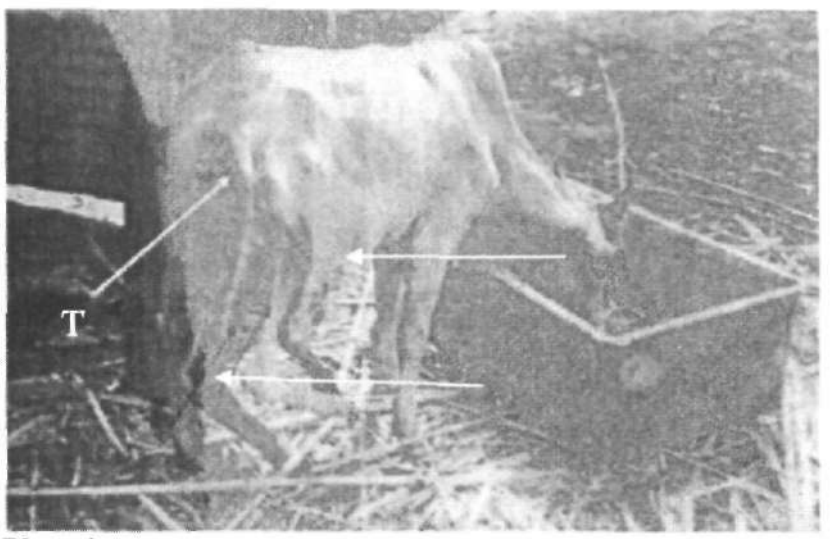


Plate 1: Photograph of the bull with notomelia. Note the two extra hind limbs (white arrows), the deviated tail (white arrow T) and a normal patent anus adjacent to the tail on left and a hidden atresia ani beneath the tail.

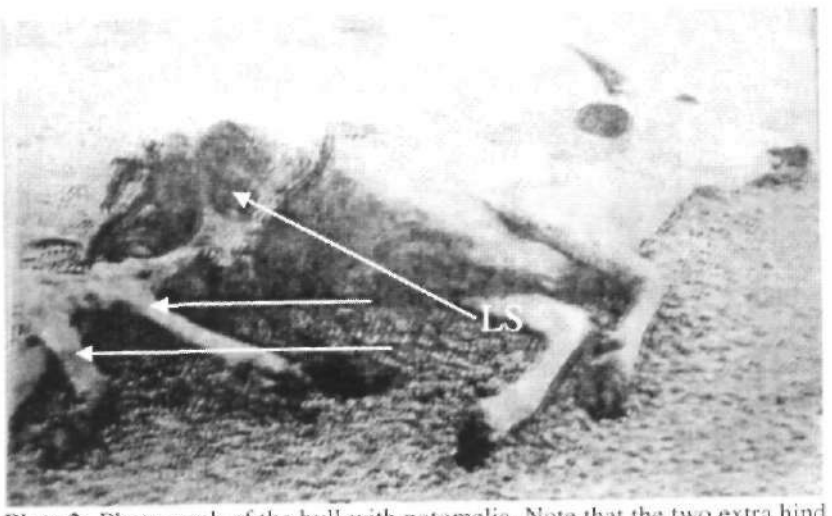


Plate 2: Photograph of the bull with notomelia. Note that the two extra hind limbs (white arrows) were at the lumbosacral (white arrow LS) region on the right.

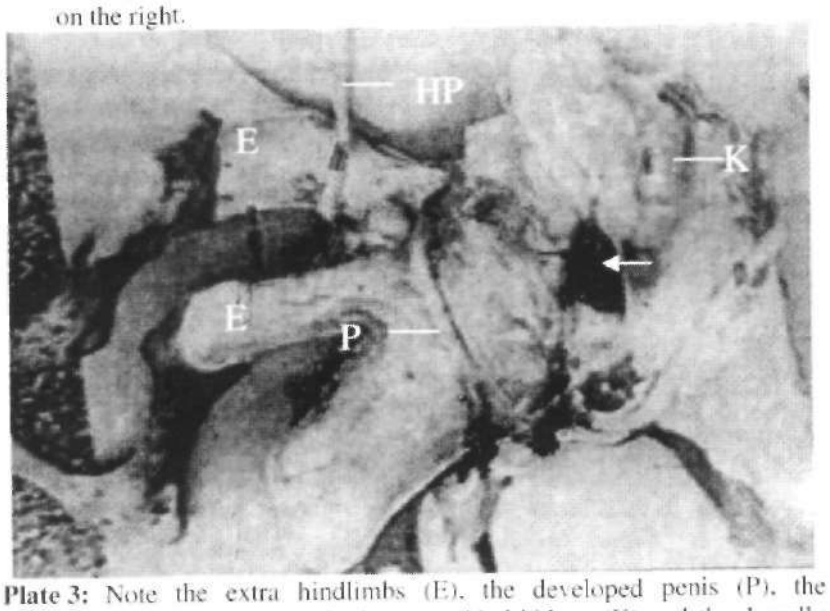


Plate 3: Note the extra hindlimbs (E), the developed penis (P), the hypoplastic penis (HP), the hypertrophied kidney (K) and the dorsally attached intrapelvic urinary bladder (white arrow) of the bull.

Conclusion

In conclusion, the congenital defects observed in this bull were severe, and could result in impairment of its normal reproductive capacity and rendered it unfit for breeding. Traumatic reticulitis (Traumatic reticulopericarditis) was the most likely cause of the death of this animal.

The general possible aetiologic agents of congenital malformations were reported to include teratogenic environmental factors, hereditary (genetic), embrayonic duplication, dispersing of germinal cells, splitting of embryo, over ripe oocysts, some types of viruses, toxins, plants, drugs (Dennis,1975; Schonfelder *et al.*, 2003; 2004) radioactive substances especially at war zones and mining industrial areas. Congenital defects generate a lot of interest, curiosity, superstitious believes and very serious diverse socioeconomic problems in animal breeding and human (Ibrahim *et al.*, 2010).

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