Sero-epidemiological and clinico-pathological studies on some infectious & parasitic diseases in wild ungulates and their relationship with livestock

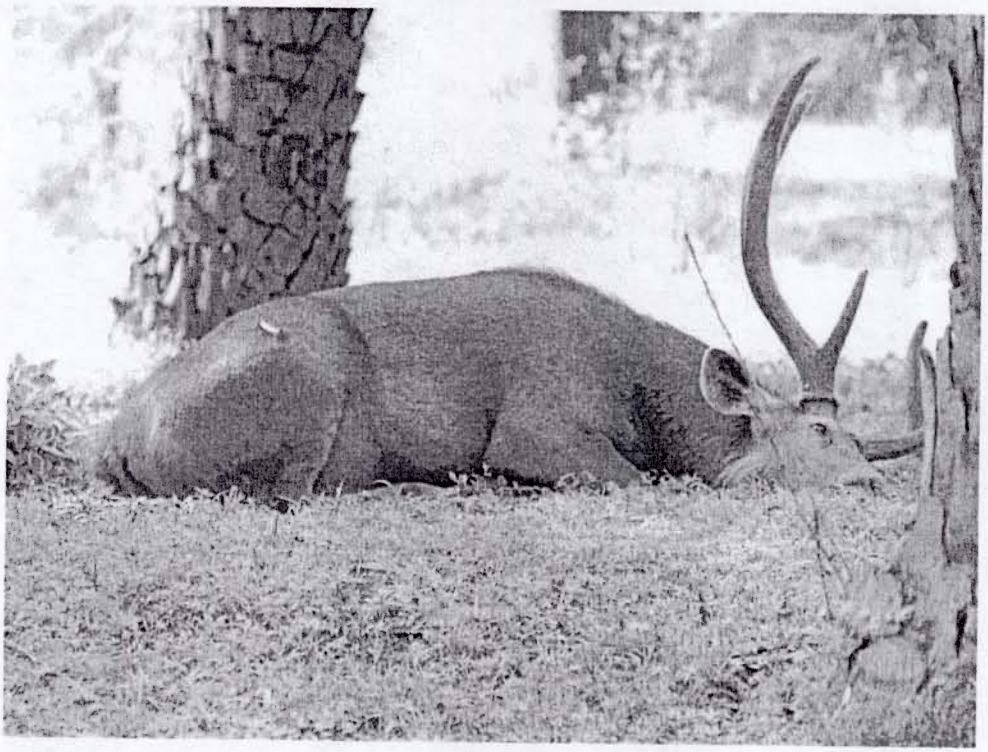

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Plate 18.3

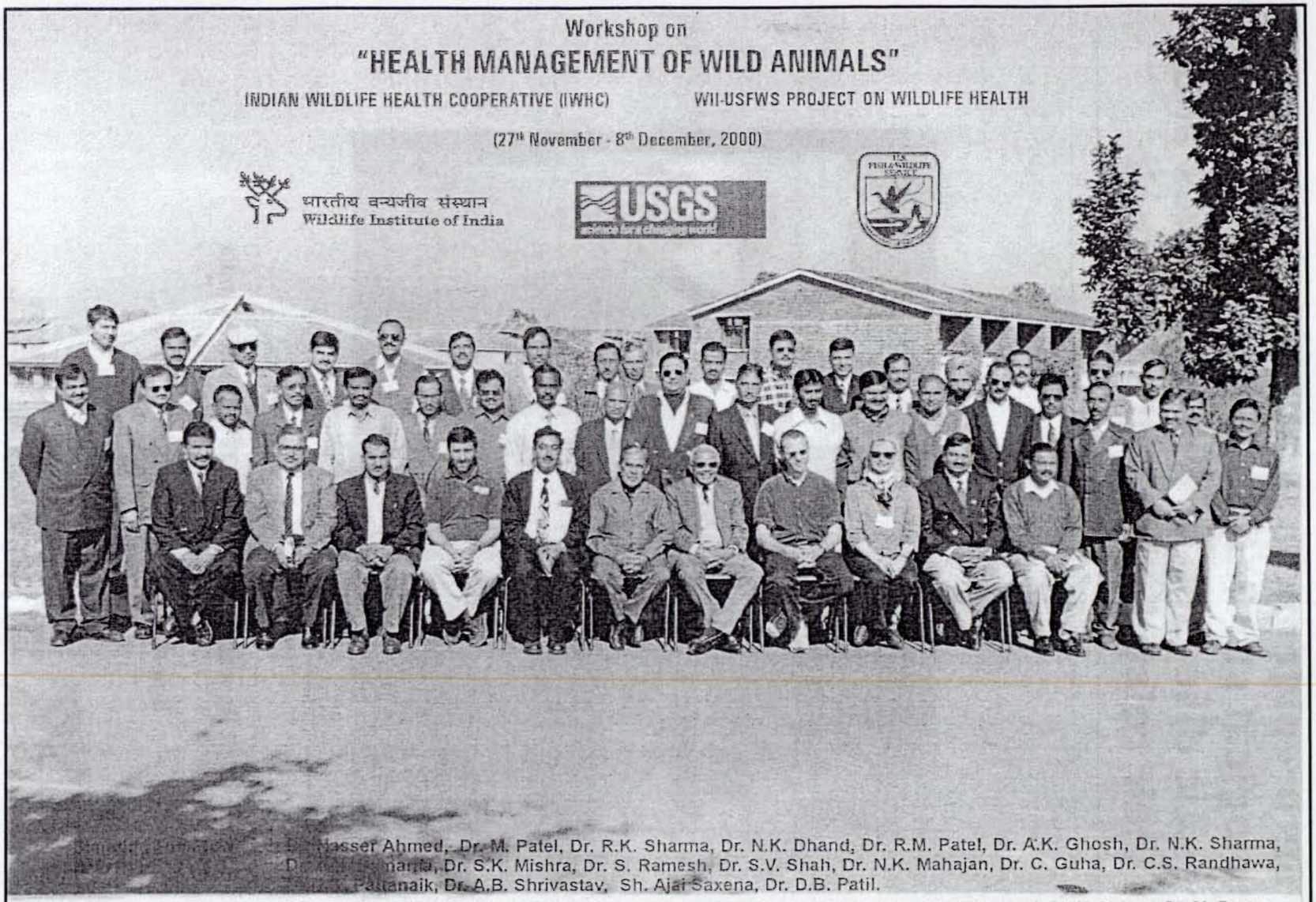

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Table of Content

List of Tables

List of Appendices

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CHAPTER 1

INTRODUCTION

Sariska Tiger Reserve was established in 1978. The present area of the reserve is 866 Sq. Km. The area lies in the Alwar district of the Rajasthan state of India. The forest type is tropical dry deciduous thorn forest. The terrain is undulating plateau lands and wide valleys.

Major forest includes Dhok (*Anogeissus pendula*) which is dominant tree species covering over 90 percent area of the forest. *Boswellia serreta* and *Lannea coromandelica* grow at rocky patches. Kattha (*Acacia catechu*) and Bamboo are common in the valleys. Some valleys support Palas (*Butea monosperma*) and Ber (*Zizyphus spp*). Besides these, some noteworthy tree species are Arjun (*Terminalia arjuna*), Gugal (Commiphora wightii), Kadaya (*Sterculia urens*), Amla (*Emblica officinalis*), Bahera (*Terminalia bellerica*).

Major fauna includes leopard, caracal, rusty spotted cat, jungle cat, four-horned antelope, sambar, cheetal, wild boar, blue bull, jackal and hyena.

According to 1991 census, there were 10, 344 people living in 24 villages in the core zone and 2, 43, 667 people in 246 villages in the buffer zone of the tiger resource.

Livestock population in the core zone is estimated around 35, 396 and 1, 42, 998 in the buffer zone. Cattle rearing is the main profession of local people and they depend upon forest areas for grazing. The population of the livestock is increasing steadily. Thus, core as well as buffer zones experience heavy burden of grazing and interface conflict between wildlife and domestic animals.

Foot and Mouth Disease (FMD), Haemorrhagic Septicaemia (HS), Bluetongue (BT) are most important endemic disease of livestock in the area. In the year 1970-71, approximately 1500 sambar deer died due to haemorrhagic septicaemia thought to be originated in nearby livestock.

Wildlife and domestic animals share many infections that may be transmitted in any direction. In India, where as considerable progress has been made in the control and prevention of diseases of livestock, there has not been any systematic study and interest in diseases of wildlife and their role in population dynamics of wildlife species. At present there is paucity of information and published material on wildlife diseases.

Considering the above factors, Sariska Tiger Reserve was thought to be an ideal place for a pilot study on wildlife diseases mainly through serological investigations with following objectives :

1. To define presence or absence of selected diseases in wild ungulates and livestock in and around Sariska Tiger Reserve.

2. To establish physiological reference values of wild ungulates.

CHAPTER 2

METHODOLOGY

2.1 Introduction

Biological samples were collected from free-ranging sambar deer and surrounding domestic ruminants including buffaloes, cattle, sheep and goats. Various samples collected from these animals were subjected to different tests namely haematology, serum biochemistry, faecal examination for gastro-intestinal parasites, blood smear examination for blood protozoan parasites, microbiological studies on rectal and nasal swabs and serological studies for detection of antibodies against infectious diseases.

To achieve the task of collection of samples, processing, preservation and storage and transportation to specialized labs as well as immediate examination of samples, a field laboratory was established at the study site in Sariska Tiger Reserve. The laboratory was equipped with a electronic balance, incubator, hot air oven, autoclave, centrifuge, liquid nitrogen containers, refrigerator, microscope, glassware, plasticware, chemicals and reagents. The lab was equipped for performing faecal examination, haematological examination, microbiological examinations (primary culturing and isolation of bacteria for further studies) as well as for serum separation, preservation and storage for transportation to specialized laboratories for biochemistry and serology. The medicines and drugs alongwith darting equipment were also present in the lab for conducting immobilization operations.

2.2 Field immobilization of wild ungulates

Following five areas were selected for immobilization and collection of samples from sambar deer.

1. Kalighati waterhole
2. Feta ki pal
3. Khajuron wala nala
4. Brihmnath waterhole
5. Tarunda waterhole

In all, 33 wild ungulates were captured, which included 32 Sambars (*Cervus unicolor*) and one cheetal (*Axix axis*). The cheetal was captured physically without chemical

immobilization. Care was taken not to recapture the same animal twice by marking the animals inside the ear-pinna.

Immobilizations were conducted generally in the afternoons baring a few exceptions when animals were captured in the late forenoon. After spotting the animal in herd, we waited for the animals to come in proper posture to ensure a good access to the rump or shoulder region of the animal for darting. Before darting *i.e.* before administering the immobilizing agent, the stress on the animal was evaluated qualitatively. If for certain reasons, the animal was too excited or frightened, the decision for darting the animal was adjourned. The ambient temperature and weather conditions were recorded. A proforma for recording of the immobilization data is given as Appendix 3.

The General Health/ Body Condition Evaluation (BCE) of the animals were judged on the basis of following chart:

Body Condition Evaluation

Body part	**Point=0**	**Point=1**	**Point=2**
Skin coat	smooth with lustre	Dull without or with little lustre	Rough, thick with folding, no lustre
Flank	Depression is barely visible, Outline is indistinct	Slightly concave and outline visible	Depression concave and tucked in
Ribs	Thoracic surface is smooth, ribs not visible	Ribs are visible but all can not be counted	Ribs clearly visible with distinct intercostal depressions
Pelvic girdle	Bony projections of pelvic girdle are barely visible	Slightly visible	clearly visible
Vertebral column	laterally it is smooth without any break, Lumbar processes invisible	lateral processes of the lumbar are visible but not prominent	lateral processes prominent, dorsal processes of vertebrae seen
Lumbar shelf	No depression in shelf, Appears almost round from behind	Slight depression on either side	Depression deep and concave

Interpretation

0-4	-	Good
5-8	-	Fair
9-12	-	Poor

Time and route of injection of immobilization and reversal (antagonist) agents were recorded. Degree and quality of sedation were evaluated as no effect, insufficient, moderate and deep sedation. Respiration rate, rectal temperature and pulse rate were recorded during the immobilization. Induction as well as reversal time were recorded and quality of reversal was noted. The animals were followed for some time, without coming directly into the sight of the animal until the animal restored normal activities and appeared to be 'fully alert'. This was especially taken into account in cases where recovery was not considered complete.

Following agents were used for immobilization :

i. Xylazine hydrochloride (Rompun® 500mg) (Bayer, Leverkusen, West Germany).

ii. Ketamine hydrochloride (Ketaset® 100mg/ml) (Fort Dodge Laboratories Inc., Fort Dodge, Iowa USA).

iii. Medetomidine hydrochloride (Zalopine® 10mg/ml) (Orion Corporation, Farmos Group Ltd., P.O.Box 425, SF - 20101, Turku, Finland).

The reversal of the immobilized animals was achieved using following agents:

i. Yohimbine hydrochloride (Reverzine® 10mg/ml) (Parnell Laboratories, Australia 6/476 Gardeners Road, Alexandria, NSW 2015).

ii. Atipamezole (Antisedan® 5mg/ml) (Orion Corporation, Farmos Group Ltd., P.O.Box 425, SF - 20101, Turku, Finland).

The immobilizing agents were administered using projectile syringes and a CO_2 - powered tele-injection equipment (Telinject Vario 4V, Telinject, Romerberg, West Germany). Injections of reversal agents were given using hand held disposable syringes via intravenous (IV) or intramuscular (IM) route.

Initially, a combination of xylazine and ketamine was used to sedate four sambar deer. After seeing the quality of sedation and difficulties faced during induction as well as in reversal, a combination of medetomidine and ketamine was attempted. The dose rates and routes are presented in Table 1. For reversal of xylazine+ketamine, yohimbine was used whereas medetomidine+ketamine sedation was reversed using either atipamezole or yohimbine.

Table 1 Doses & routes of immobilization drugs

No. of animals	Immobilizing agent	Average dose (mg)	Route	Reversal	Average dose (mg)	Route
4	Xylazine+ketamine	340+100	IM	Yohimbine	55	IV
14	Medetomidine+ketamine	9+160	IM	Atipamezole	25	IV/SC
13	Medetomidine+ketamine	9+160	IM	Yohimbine	60	IV
1	Medetomidine+ketamine	9+160	IM	Yohimbine	50	IV
				Atipamezole	15	IV/SC

Parameters recorded during immobilization of these non-domestic free-ranging ungulates included, sex, approximate body weight of the animal, whether the animal was present in herd or alone, ambient temperature, weather conditions *e.g.* whether the day is bright or cloudy, physical condition and emotional state of the animal before darting, time of injection, induction period and reversal time as well as behaviour of the animal during the operation. Other parameters included were rectal temperature, pulse rate (from caudal artery)/ heart rate using a stethoscope, respiration rate and the quality as well as physical condition of the animal. Any other symptoms shown during the sedation like convulsions or tremors, salivation, bloat etc. were also recorded. The animals were treated symptomatically, whenever required. Any other special finding or experience gained during the immobilization was also recorded.

After each darting, the animal was observed till it was recumbent without coming in its direct sight. On induction, the animal was quietly approached and blindfolded by covering the eyes using a thick, dark cloth. The animal was monitored clinically for the degree and quality of sedation and then the samples were drawn.

2.3 Collection of samples

2.3.1. Blood:

a. Blood was drawn from the jugular vein in plain Vacutainer® (Becton Dickinson VACUTAINER Systems, Rutherford, New Jersey - 07070, USA). The blood samples were allowed to clot without disturbing them to obtain serum for estimating biochemical parameters and serological examinations.

b. Blood samples were also collected in heparinized Vacutainer® to obtain unclotted blood to be used in haematological examinations.

c. Blood smears were made immediately taking the blood directly from the vein and were allowed to dry in air. The smears were, then, fixed with acetone-free methanol in the field itself before taking to the lab for examination for haemoprotozoan parasites.

2.3.2. Faecal pellets were collected in clean glass vials with preservative (10% formal saline). The pellets were also collected/ dissolved in normal saline for immediate examinations in the field lab. Pellets in 10% formal saline were stored for onward transmission to specialized labs for further examination.

2.3.3. Nasal and rectal swabs were also collected in Amies Transport Medium with charcoal (Precision Dynamics Corp., 13880 Del Surf Street, San Fernando, California, USA) for microbiological studies. These were kept at room temperature till processed in the lab.

After collection of the samples, the animals were revived by injecting antagonist as described earlier. In most animals, Selevite-E (combiantion of Selenium and Vitamin E) was administered intramuscularly @ 3-5 ml/animal as supportive therapy before reversal. As soon as the animal became fully alert, collected samples were rushed to the field lab immediately for processing, storage and examination.

2.3.4 Sample processing, storage and analysis at the field laboratory

Sera were removed from the clotted blood by centrifuging them at 2000 rpm for 10 min at room temperature. The serum samples were then stored at -196°C in liquid nitrogen in cryovials (1.8ml capacity) and properly marking their identity. The serum

sample was used in serum biochemical and serological studies. Multiple copies of each serum sample were made for use in various labs.

Blood samples, in heparin, were thoroughly mixed and used immediately for studying routine haematological parameters. Faecal pellets were examined in the field lab for endoparasite eggs and larvae. Preserved faecal pellets in 10% formal saline were stored at room temperature till transportation to specialized labs. Rectal and nasal swabs were processed by primary culturing and isolation for identification of bacteria in the field lab. Certain swabs and isolated bacteria were then taken to specialized labs for further studies.

2.4 Laboratory analysis

2.4.1. Faecal examination: This included gross examination and microscopic examination of the collected faecal pellets. Fresh faecal samples were collected in the morning hours, usually between 8 to 9 a.m. Moreover, samples were also collected from the immobilized animals directly from the rectum. Samples were collected in clean glass vials containing sufficient quantity of normal saline and 10% formal saline. Faecal samples were, thereafter, examined in the field laboratory and also referred to the specialized laboratory at College of Veterinary Sciences, CCS HAU, Hisar and College of Veterinary Sciences, GBPUA&T, Pantnagar for thorough examination and confirmation.

In all, about 450 samples from various domestic and wild ungulates comprising of spotted deer (Cheetal) (100), Sambars (150), sheep and goats (100) and cattle and buffaloes (100) were collected and examined. The samples were examined using the direct examination method, sedimentation method and floatation technique.

i) Direct smear examination method

The direct smear was made by using either tap water or physiological saline. The saline is good for trophozoids, as they will remain intact and motile. This is a rapid and an easy method but eggs and oocysts are not concentrated. A drop of the diluting fluid was placed on a glass slide and thoroughly mixed with a bit of faeces. It was

covered with cover slip and tilted slightly in order to allow the lighter eggs to flow away from heavy debris. The smear was then examined under light microscope.

ii) Sedimentation method

Most operculated trematode eggs and a few nematode eggs are difficult or impossible to recover without flotation and sedimentation technique. One gram of faeces was mixed with 40 ml of saline in a beaker, making certain that the faeces were thoroughly dissociated/homogenized to make the eggs separate. The homogenized faecal pellets were then filtered through a sieve. Filtrate was later centrifuged at a moderate rate of 2 to 3 thousand rpm for 5 minutes. The supernatant was discarded and a small quantity of sediment was collected with the help of a pipette. A drop of this sediment was put on a clean slide, covered with cover slip and examined under the microscope.

iii) Floatation method

One to two grams of faecal sample was taken with sufficient saline in a beaker to make a fluid mixture. The faeces must be dissociated from the eggs and oocysts. The mixture was filtered through muslin cloth and the debris was discarded. The fluid was transferred in centrifuge tubes and centrifuged for 5 minutes at 1000 RPM. The supernatant was discarded and Zinc Sulphate solution or saturated salt solution was added and mixed thoroughly. It was centrifuged again for same time at same speed. Using the wire loop, fluid was removed from the top of the centrifuge tubes, placed on a clean glass slide, covered with coverslip and examined under microscope for lighter eggs. The lighter eggs float due to the differences in specific gravity of the medium in which the eggs get suspended.

2.4.2 Haematological examination

Haematological examinations were conducted by standard techniques as described by Coles, 1986. For haematological study following parameters were taken into consideration:

1. Total erythrocyte count (TEC)
2. Haemoglobin estimation
3. Packed cell volume (PCV)

4. Total leucocyte count (TLC)
5. Differential leucocyte count (DLC)
6. Erythrocytic indices
7. Erythrocyte sedimentation rate

1. **Erythrocyte Sedimentation Rate (ESR) :** The ESR was determined by Westergren method and was recorded after 1 hour. Unit : mm/1hr.

2. **Haematocrit:** Haematocrit or Packed Cell Volume (PCV) was calculated by Macrohaematocrit (Wintrobe) method. Unit : %.

3. **Haemoglobin Concentration:** Haemoglobin concentration in the blood was determined by acid haematin (Sahli) method using Sahli haemoglobinometer. Units : g%.

4. **Enumeration of Formed Elements: Total Erythrocyte Count (TEC)** and **Total Leucocyte Count (TLC)** were performed by haemocytometry on the modified double Neubauer's grid. The counts are expressed in million cells per µl (TEC) and number of cells per µl (TLC), respectively.

5. **Red Blood Cell Indices:** The quantitative measurements of the average, size haemoglobin content and haemoglobin concentration of the erythrocyte were done by calculating commonly used indices of erythrocyute viz. **Mean Corpuscular Volume (MCV), Mean Corpuscular Haemoglobin (MCH) and Mean Corpuscular Haemoglobin Concentration (MCHC).**

6. **Differential Leucycyte Count (DLC) and Cell Morphology:** A blood smear was made just after the collection of blood and fixed with acetone free methanol following air-drying it. The smear was stained by Geimsa's stain and was examined under oil immersion lens (1000x) for counting different types of lecucocytes. The number is expressed as the % of total leucocytes.

   Any abnormalities in the morphology of red or white cells like variation in the colour, size and shape and presence of inclusions were noted.

2.4.3 Serum biochemistry

The serum samples were transported to the lab in liquid nitrogen for estimating levels of blood biochemicals. In all 20 parameters of serum biochemistry were performed. Sodium and potassium were assayed by Ion Selective Electrodes in AVL-9120 Electrolyte analyzer. Rest of the biochemistry was done on Seac CH-100 semi-autoanalyzers using commercially available kits (M/s Miles India Ltd., Baroda, India for chloride, SGOT, SGPT, blood urea, calcium, phosphorus, total protein, albumin, CPK, serum bilirubin, cholesterol, and serum creatinine; M/s Lupin Laboratories Ltd., Bombay, India for serum glucose and alkaline phosphatase; M/s Ranbaxy Laboratories Ltd., New Delhi, India for uric acid and I-GT and M/s Span Diagnostics Ltd., Sachin, India for LDH). The End-point reaction was involved in the essay of Glucose (GOD-POD), Chloride, blood urea, calcium, inorganic phosphorus, total protein, albumin, serum bilirubin, cholesterol, and uric acid (uricase-PAP method) whereas estimation of LDH (pyruvate to lactate method), alkaline phosphatase (PNP method), I-GT (Szasz method), SGOT (SCE method), SGPT (SCE method) and CPK involve kinetic reaction. Creatinine estimation by picrate method utilises fixed time reaction.

2.4.4 Serological examination

i. Microscopic Agglutination Test (MAT) for Leptospirosis

The test was done as per the method described by Wolff (1954). A battery of well grown cultures of reference strains of *Leptospira interogans* serovars, that represent all known serogroups (approximately 20), were prepared to cover all possible types of *Leptospira* infection. Alternatively, cultures of strains of *Leptospira interogans* in liquid medium could be used, representing all serogroups known to be prevalent in the locality. Live cultures were used, although cultures killed by the addition of formalin (0.5% w/v) neutralized with magnesium carbonate could also be used. Killed suspensions are more convenient and safer for routine work and they may be stored for a month or longer before use but they may cause non-specific agglutination reaction because of presence of traces of formic acid.

The test proper is described as under:

1. The serum sample was diluted serially from 1 in 5 to 1 in 15000 by dropping technique. The procedure is described in the following table. This provided a series of dilutions of 1 in 10, 1 in 30, 1 in 100 *etc.* to 1 in 3000 or more.

	Number of drops of stated reagent added to well number					
Reagent	**1**	**2**	**3**	**4**	**5**	**6**
First row (dilution of serum)						
Saline (drops)	8	9	9	none added	none added	none added
Serum (drops)	2	1[a]	1[b]	none added	none added	none added
Initial serum dilution	5	50	500	--	--	--
Second row (test proper)						
Saline (drops)	none added	2	none added	2	none added	2
Serum 1 in 500 (drops)	none added	None added	none added	none added	3	1
Serum 1 in 50 (drops)	none added	None added	3	1	none added	none added
Serum 1 in 5 (drops)	3	1	none added	none added	none added	none added
Culture (drops)	3	3	3	3	3	3
Final serum dilution	10	30	100	300	1000	3000

a : Serum diluted 1 in 5 from well number 1

b : Serum diluted 1 in 50 from well number 2

2. The mixtures were incubated at 37°C for 3 h and allowed to stand at room temperature for 1 h before reading. The test mixtures can be held at 4°C overnight, if required to be read the following morning.

3. A no serum control was included to ensure that the antigens were satisfactory.

4. To read the test, a drop of the antigen-serum mixture from each well was placed on a microscopic slide. The drops were examined at 100-200x magnification using darkfield illumination. It was not necessary to place a coverslip over the drop.

   Agglutination of living leptospires appeared as lightly refractile, spherical masses. Lysis, which was previously thought to occur, did not take place. With formalinized cultures, agglutination appears as loose, irregular cotton-wool-like clumps.

5. The highest dilution of serum was detrmined by the dilution of serum which agglutinated 50% or more of leptospires in the drop of suspension. This was judged by the proportion of organisms free between the agglutinated clumps. It was compared with the no serum control. The dilution represented the titre of antibody specific for the particular serovar used.

Interpretation : The test is essentially sero-group specific but many serovars are related serologically through their minor antigens and there is a certain amount of cross reaction between the various strains used as antigens, especially in the early stages of the infection. It may not be possible to obtain a true indication of the serogroup of the infecting strain at that stage. Later specimens, however, tend to react mainly with the strain that represents the serogroup to which the infecting strain belong. A series of specimens of serum taken over a period of several weeks will result in a demonstrable rise in the titre of the homologous antibodies which confirms the diagnosis of present infection. The strain that reacts most strongly with the late specimens indicates the serogroup of the infecting strain. However, in the present study, as it was not feasible to obtain a paired serum samples, a titre of 1 in 100 or more was considered as positive for a specific serovar.

ii. Agar Gel Precipitation Test for Bluetongue virus

The BTV specific antibodies were tested by agar gel immunodiffusion test as described earlier (Jochim and Chow, 1969). One percent agar in phosphate buffer was pepared and poured in petri plates. After solidification of agar, wells were cut in it using templates. The wells were then filled with antigen (central well) and antisera (test sera, in peripheral wells), using separate clean Pastuer pipettes. The plate was covered and incubated in damp chamber at 37°C . The plates were examined after 24 h and later for the precipitin lines.

iii. Monoclonal Antibody Competitive ELISA

Lower titre of antibodies in the test sera may not permit higher dilutions of the test sera and therefore serum protein may adhere non specifically and thus falsely recorded as antibodies by the antispecies-enzyme conjugate. To overcome this disadvantage, competitive blocking ELISA (Sorensen *et al.*, 1992) has been described, which was used to detect the specific antibodies to RNA polymerase antigen (VIA antigen) in the sera. In this procedure, the antigen is immobilised by the trapping antibodies. Serial dilutions of the test sera are made and allowed to interact with the antigen. Another high titred antisera raised against the purified antigen in laboratory animal is then added. In the present study, monoclonal antibodies against RNA polymerase antigen (VIA antigen) were used. This antisera is referred as laboratory sera. More antibodies in the lower dilutions of the test sera would bind to more sites on the antigen and thus laboratory sera would find fewer sites on the antigen to bind. Subsequently, upon addition of enzyme conjugated antisera raised against sera of that laboratory animal would also fail to bind and thus little colour could only develop. As the antibodies in the test sera are diluted, more sites on the antigen would remain available for the laboratory sera to bind and thus more colour would develop. From the optical densities, percentage inhibition is calculated by the formula-

Percentage inhibition= Maximum OD-TestOD /Maximum ODX100.

The test sera dilution giving 50% inhibition is taken as the titre.

iv. Rose Bengal Plate Agglutination Test

This test is used for detection of *Salmonella* antibodies in serum samples of human patients and animals. It is a simple and rapid test and can be carried out in the field.

Materials required

1. Rose Bengal Test (RBT) antigen.
2. Serum samples.
3. Micro pipettes, 10-100 l and 2-20 l.
4. Clean white tiles or glass plates.
5. Wooden tooth picks.

Stepwise procedure

1. Drop out 0.04, 0.02, 0.01 and 0.005 ml of the serum sample with the help of micro pipette on white tiles.
2. Drop out 0.02 ml of RBT antigen and mix with the serum samples with tooth picks. Rock the tile back and forth for 4 min.
3. Look for agglutination reaction.

Observations and interpretation of results

Positive reaction- Fine agglutination/ course clumping with clearing.

Negative reaction- Complete absence of agglutination.

2.4.5 Examination of blood smears for protozoan parasites

Unstained and Giemsa stained blood smears were thoroughly examined for various blood protozoan parasites including Trypanosomes, Theilaria, Babesia and Toxoplasma.

2.4.6 Microbiological studies

Rectal and nasal swabs were collected from 32 free-ranging sambars (*Cervus unicolor*) after immobilization at Sariska Tiger Reserve, Sariska, Alwar (Rajasthan). The swabs were brought to the laboratory in modified Amies Transport Medium with charcoal and kept at room temperature till processing for isolation was done. For isolation, buffered peptone water, modified McConkey lactose agar and eosin-methyline blue (EMB) agar (HiMedia, Mumbai) were used as enrichment, differentiation and selective media, respectively. The smooth, moist colonies having metallic sheen on EMB agar were randomly picked up and processed for isolation and identification. The isolates were identified on the basis of their cultural, morphological and biochemical characterisitcs (Cowan and Steel, 1975). These were serotyped at the National *Salmonella* and *Escherichia* Centre, Central Research Institute, Kasauli (H.P.), India. Antibiogram of all isolates were determined for 11 antimicrobial agents, namely nalidixic acid, cotrimoxazole, ciprofloxacin, norfloxacin, chloramphenicol, kanamycin, oxytetracycline, streptomycin,

furazolidone, cephlaxin and neomycin, employing disc diffusion method of WHO (1961).

i. Congo Red Dye Adsorption Assay (CRDA)

The test was performed by inoculating the isolates on trypticase soy agar (TSA) [pH 7.3] containing 0.03% Congo red dye (Ishiguro *et al.*, 1985). After 18 and 36 h of incubation at 37°C, colonies were examined. Appearance of brick red colonies indicated the adsorption of dye by bacteria.

ii. Klebobiocin assay, Surface anionicity and Sephadex binding

Sensitivity to Klebobiocin produced by *Klebsiella aerogenes* (G4, 52K) strains was determined for all isolates according to Singh (1997). Briefly, Klebobiocin producing G4 and 52K strains were grown on BHI agar plates for 24 h at 37°C. One loopful of klebocin producer strain was point inoculated on a freshly swab inoculated BHI agar plate with test strain; incubated at 37°C for 24 h and observed for appearance of clear zone of growth inhibition around the point of inoculation.

Surface anionic charge of the isolates was measured by binding of bacteria to DEAE Sepharose (Singh, 1997). Test bacterial strain grown in BHI broth (18h at 37°C) was centrifuged to harvest cell pellet. Bacterial cells were washed twice with normal saline solution and resuspended in the same to the 50% absorbance level at 540nm (A540). Then 2ml of bacterial suspension was mixed with 200µl of 2% (v/v) DEAE sepharose and incubated at shaker platform (200rpm) for 30 min. It was allowed to stand for 30 min at 25°C. Thereafter 200µl of supernatant was taken for A540 reading. Test was conducted in triplicate and anionicity was calculated as follows:

$$\text{Anionicity} = \frac{0.5 - \text{A540 of test}}{0.5} \times 100$$

Preparations of *Salmonella* Gallinarum strain with surface anionicity value of 50 was used as positive control. Similarly, Sephadex binding was determined for these isolates.

iii. Salt aggregation test

Salt aggregation was performed according to Rozgaonyi *et al.* (1985). Briefly, overnight cultures were washed twice in 0.002 M sodium phosphate buffer (pH 6.8)

and resuspended to a 10 percent transmission using 620 nm wavelenth. Of this suspension, 50µl were mixed with equal volumes of ammonium sulphate (2.0, 1.5, 1.25, 1.0, 0.5 and 0.25 M), to give final volumes of 1.0 M, 0.75 M, 0.625 M, 0.5 M, 0.25 M and 0.125 M, respectively. A visible cell aggregation within five minutes was regarded as a positive reaction. A bacteial suspension in plain buffer served as a control. The lowest final ammonium sulphate molarity which gave visual bacterial cell clumping was scored as the value for bacterial surface hydrophobicity.

iv. Hexadecane adherence assay

Adherance to n-hexadecane (Sigma) was assayed using the method described by Greene *et al.* (1992). Washed cells (late exponential phase growth) were suspended in phosphate buffered saline (PBS, pH 7.5) to an absorbance (A540) of 0.5 at 540nm (Spectronic 20, Bausch and Lomb). To 3 ml of this suspension, 0.8 ml hexadecane was added. The contents were mixed (30 seconds) and after phase separation (20 minutes at room temperature), A540 of the aqueous phase was measured. Bacterial suspensions without hexadecane were used in measure the initial A540. Samples were run in duplicate and the final adherence value was calculated using the mean. The percentage of bacteria that adhered to hexadecane was expressed as:

$$\frac{\text{A540 (initial) - A540 (after adherence)}}{\text{A540 (initial)}} \times 100 = \% \text{ Adherence}$$

Adherence was considered positive (++) if the adherence was over 75 percent, intermedate (+) if 25 to 75 percent, and negative (-) if under 25 per cent.

v. Cytotoxicity assay

The cell free culture filtrates were prepared by the method of Evans *et al.* (1973). These were tested on monolayer of Madin Darby Bovine Kidney (MDBK) cell line at 37°C and observation were recorded after 24h of incubation.

vi. Pathogenicity

Pathogenicity of the isolates was examined by mice-lethality test. The isolates were propagated in BHI broth (HiMedia) and 0.1 ml of each of 6h-old-culture was administered intra-peritoneally in adult mice (1½ -10 month-old). The animals were observed regularly for 72h for any change. Mice found dead during this period were

opened and lesions were observed. The mice which were depressed and having diarrhoea were considered morbid.

2.4.7 Post mortem examinations

Occassional cases of deaths in domestic livestock and wild animals were observed during the period of study at Sariska Tiger Reserve. The findings in such individual cases added to the understanding of existence of a disease in clinical form. Necropsies were conducted in the field with all precautions. Utmost care was taken in keeping the area and the surroundings hygienic. The cases included a buffalo calf, a buffalo, sambars and spotted deer. The samples were then taken to specialized laboratories for histopathological examination in an attempt to ascertain the cause of death in each case.

CHAPTER 3

RESULTS

The study involved examination and assessment of general body condition of the common ungulates in different seasons, hematological and serum biochemistry values for apparently healthy sambar and cheetal deer *vis a vis* domestic ruminants in and around Sariska Tiger Reserve and serological prevalence of common bacterial, viral and heamoprotozoan diseases. The diseases included leptospirosis, salmonellosis, bluetongue, foot and mouth disease and babesiosis. The microbiological studies involved the isolation of bacteria from nasal and rectal swabs followed by characterization of isolated cultures and their pathogenicity studies. The results for these parameters are presented in various tables. The data on drugs, along with dosage and routes, used for immobilization, revival and supportive treatment for captured animals for sample collection as well as the clinical monitoring parameters are also summarized in this chapter. These results are presented hereunder:

3.1 General Health /Body Condition Evaluation of the wild animals in various seasons is summarized below:

Overall body condition of ungulates in Sariska Tiger Reserve during the study period in different seasons

Season	Nilgai	Sambar	Cheetal
Winter	Fair	Good	Good
Summer	Good	Fair	Fair
Rainy	Fair	Good	Good

3.2 Immobilization

Table 2. Summary of anesthetic drugs used for field immobilization of wild ungulates

Immobilization			Reversal		
Drug(s) used	**Dose (mg) & Route**	**Average induction time**	**Drug(s) used**	**Dose (mg) & Route**	**Average reversal time**
Xylazine + Ketamine (4)	340+100 IM	5.5 min	Yohimbine (4)	55 IV	5.75 min
			Atipamezole (14)	25 IV + SC	3 min
Medetomidine + Ketamine (28)	9+160 IM	7-8 min	Yohimbine (13)	10 IV	4.5 min
			Yohimbne + Atipamezole (1)*	60 +15 IV+SC	13 min

Figures in the parentheses indicate the number of animals

* Animal could not fully recover by Yohimbine and later Atipamezole was used and animal could revert after a total gap of 13 min.

3.3 Clinical parameters

Table 3. Clinical parameters of immobilized sambars

Animal	Sex	Body condition*		Pulse rate (per min)	Respiratory rate (per min)	Temperature (°F)
S-1	M	GOOD	2	-	20-40	101.5
S-2	M	FAIR	5	-	-	-
S-3	F	FAIR	5	42	32	-
S-4	M	FAIR	5	-	-	-
S-5	F	FAIR	6	-	36	-
S-6	F	GOOD	4	70	40	102
S-7	M	GOOD	2	50	20	102
S-8	M	GOOD	3	60	18	102.5
S-9	F	GOOD	3	50	16	102.4
S-10	M	GOOD	3	60	36	102.4
S-11	M	GOOD	1	60	20	103.5
S-12	F	GOOD	2	-	20	103
S-13	M	GOOD	2	68	22	101.8
S-14	F	GOOD	3	70	18	102.2
S-15	F	GOOD	1	68	25	101.5
S-16	M	GOOD	2	60	20	102.5
S-17	F	GOOD	1	64	16	103
S-18	M	GOOD	3	60	22	102.5
S-19	M	GOOD	3	68	20	102.5
S-20	M	GOOD	2	62	18	101.5
S-21	M	GOOD	2	64	20	-
S-22	M	GOOD	3	64	16	103
S-23	M	GOOD	2	68	16	101
S-24	M	GOOD	2	-	16	102
S-25	F	GOOD	3	62	16	101.8
S-26	M	GOOD	1	72	22	102.2
S-27	M	GOOD	1	64	14	101.6
S-28	F	GOOD	1	72	22	102
S-29	M	GOOD	3	58	14	101.5
S-30	M	GOOD	1	60	12	100.8
S-31	M	GOOD	3	58	12	102.2
	M	GOOD	3	62	16	100.5
Mean				62.15385	20.83333	102.0519
Standard Error				1.376386	1.313137	0.133693
Range (Minimum-Maximum)				42-72	12-40	100.5-103.5
N				26	30	27

* Number against the condition indicates the BCE Index

3.4 Biochemistry

Table 4. Serum biochemistry values in sambar deer

Sr. No.	Parameter	Sample size (n)	Average value ± S.E.	Range
1.	Glucose (mg/dl)	32	81.56 ± 3.95	32-149
2.	Sodium (meq/L)	32	133.91 ±1.42	111-142
3.	Potassium (meg/L)	32	4.94 ±0.13	3.4-6.1
4.	Chloride (meq/L)	32	63.09 ±0.94	53-87
5.	SGOT/AST (units/L)	32	57.97 ±2.94	36-116
6.	SGPT/ALT(units/L)	32	61.65 ± 2.62	23-93
7.	Blood urea (mg/dl)	32	40.16 ± 1.97	18-64
8.	Calcium (mg/dl)	32	7.54 ± 0.24	3.7-10.1
9.	Total protein (g/dl)	32	7.48 ± 0.15	6.3-9.4
10.	Albumin (g/dl)	32	3.26 ± 0.08	2.5-5.2
11.	Globulin (g/dl)	32	4.22 ± 0.19	1.3-6.5
12.	CPK (IU/L)	32	252.97 ± 32.09	88-762
13.	Serum uric acid (mg/dl)	32	0.825 ± 0.07	0.3-26
14.	LDH (units/L)	32	564.91 ± 30.37	188-1082
15.	Inorganic phosphorus (mg/dl)	32	7.06 ± 0.33	2.4-114
16.	Alkaline phosphatase (IU/L)	32	318.78 ± 40.86	74-1153
17.	Serum creatinine (mg/dl)	32	2.7 ± 0.09	2.1-4.0
18.	Serum bilirubin (mg/dl)	32	0.4 ± 0.017	0.2-0.6
19.	Serum cholesterol(mg/dl)	32	75.56 ± 5.78	21-179
20.	γ-GTP (units/L)	29	25.62 ± 2.85	12-79

Table 5. Serum biochemistry values in cheetal deer

Sr. No.	Parameter	Value
1.	Glucose (mg/dl)	69
2.	Sodium (meq/L)	148
3.	Potassium (meg/L)	11.9
4.	Chloride (meq/L)	64
5.	SGOT/AST (units/L)	188
6.	SGPT/ALT(units/L)	77
7.	Blood urea (mg/dl)	61
8.	Calcium (mg/dl)	10.2
9.	Total protein (g/dl)	9.1
10.	Albumin (g/dl)	4.5
11.	Globulin (g/dl)	4.6
12.	CPK (IU/L)	643*
13.	Serum uric acid (mg/dl)	2.6
14.	LDH (units/L)	590
15.	Inorganic phosphorus (mg/dl)	6.4
16.	Alkaline phosphatase (IU/L)	151
17.	Serum creatinine (mg/dl)	38*
18.	Serum bilirubin (mg/dl)	1.2
19.	Serum cholesterol(mg/dl)	128
20.	γ-GTP (units/L)	128*

3.5 Haematology

Table 6. Haematological values in sambar deer

Sr. No.	Parameter	Sample size (n)	Average value ± S.E.	Range
1.	TEC (millions/µl)	32	5.91 ± 0.17	3.69-7.42
2.	Haemoglobin (g%)	32	11.68 ± 0.17	9.6-13.8
3.	PCV(%)	32	31.19 ± 0.51	25-36
4.	ESR (mm/h)	32	46.09 ± 5.30	4-112
5.	TLC (numbers/µl)	32	3359.37 ± 83.58	275-4375
6.	MCV (fL)	32	53.82 ± 1.41	41.90-74.44
7.	MCH (pg)	32	20.25 ± 0.64	15.81-34.24
8.	MCHC (g/dl)	32	37.59 ± 0.46	33.89-46
9.	DLC			
	a) Neutrophils (%)		32.59 ± 2.33	13-68
	b) Lymphocytes (%)		65.25 ± 2.35	32-87
	c) Monocytes (%)		1.22 ± 0.29	0-5
	d) Eosinophils(%)		0.84 ± 0.22	0-5
	e) Basophils (%)		0.09 ± 0.05	0-1

Table 7. Haematological values in sheep and goats

Sr. No.	Parameter	Sample size (n)	Average value ± S.E.	Range
1.	TEC (millions/µl)	55	12.06± 0.29	4.8-18.0
2.	Haemoglobin (g%)	55	10.12 ± 0.15	7.0-12.4
3.	PCV(%)	55	27.74 ± 0.58	17-40
4.	ESR (mm/h)	55	0.24 ± 0.06	0-1
5.	TLC (numbers/µl)	55	8469.09 ± 277.19	5150-14400
6.	MCV (fL)	55	2.35 ± 0.06	1.76-3.54
7.	MCH (pg)	55	0.86 ± 0.02	0.63-1.46
8.	MCHC (g/dl)	55	0.37 ± 0.01	0.31-0.47
9.	DLC			
	a) Neutrophils (%)		36.44 ± 1.20	13-63
	b) Lymphocytes (%)		60.11 ± 1.08	36-82
	c) Monocytes (%)		0.36 ± 0.08	0-3
	d) Eosinophils(%)		2.38 ± 0.25	0-7
	e) Basophils (%)		0.16 ± 0.05	0-1

Table 8. Haematological values in buffaloes

Sr. No.	Parameter	Sample size (n)	Average value ± S.E.	Range
1.	TEC (millions/µl)	56	4.87 0.13	1.8-7.6
2.	Haemoglobin (g%)	56	11.93 0.35	0.96-16.6
3.	PCV(%)	56	31.05 0.85	12-48
4.	ESR (mm/h)	56	57.45 6.61	0.140
5.	TLC (numbers/µl)	56	8962.14 372.67	4300-18500
6.	MCV (fL)	56	64.98 1.82	45.09-114.43
7.	MCH (pg)	56	25.18 0.92	2.18-53.73
8.	MCHC (g/dl)	56	38.74 0.83	3.84-48.33
9.	DLC	56		
	a) Neutrophils (%)		31.67 1.69	18-78
	b) Lymphocytes (%)		63.11 1.81	16-82
	c) Monocytes (%)		2.78 0.47	0-13
	d) Eosinophils(%)		2.93 0.39	0-13
	e) Basophils (%)		0.27 0.07	0-2

3.6 Serological examinations

3.6.1. MAT for Leptospirosis

The results of testing of various sera samples for leptospirosis using microscopic agglutination test are presented in the following table :

Table 9. Results of MAT for leptospirosis

Species	Number	Positive for leptospira serovar
Goat	G 12	patoc
	G 16	patoc
	G 22	patoc
	G 33	pyrogenes
	G 44	tarassovi
	G 45	pyrogenes
	G 46	pyrogenes
Buffalo	D 24	tarassovi
Sambar	S 1	positive using a pool of tarassovi, ballum
	S 3	and pyrogenes
	S 15	

In the present study, a total of 178 sera, collected from domestic cattle (4), buffaloes (86) and goats (55) as well as free-ranging sambars (32) and cheetal (1), were tested to study the sero-prevalence of leptospirosis. Results indicated a positivity of 12.73%) among goats, 1.16% in

buffaloes and 9.37% in sambars for different *Leptospira* serovars. Common serovars detected among wild and domestic animals were *pyrogenes* or *tarassovi*; other serovars being *patoc* and *ballum*.

3.6.2 Detection of bluetongue virus infection in wild ruminants

Our survey indicated that 2 out of 32 (6%) sambar serum samples were positive while a chital serum sample tested was found negative for BTV antibodies. In domestic livestock, 20 (38%) goats, 1 (25%) cattle and 12 (14%) buffalo serum samples were positive for BTV antibodies (Table 10). The present investigation indicated that BTV infection is prevalent in both domestic as well as wild ruminants in and around Sariska Tiger Reserve.

Table 10. Prevalence of bluetongue virus antibodies in domestic and wild ruminants in and around Sariska Tiger Reserve

Species	No. of samples tested by AGID	No. of samples positive	% positive
Cattle	4	1	25
Buffalo	86	12	14
Goat	53	20	38
Sambar	32	2	6
Chital	1	-	-

3.6.3 Mab ELISA for FMD

Following animals were found to be FMD positive in Mab- competitive ELISA where antibodies to RNA polymerase antigen (VIA) were detected in serum of animals:

Wild : C-1 (Cheetal) - one

Domestic goats : G-9, G-10, G-22, G-34, G-36, G-38, G-44, G-47, G-49-55 - Fifteen

Domestic cattle and buffalo : D-18, D34-35, D-39-40, D-42-85, D-87, D-98, D-99 – fifty two

In all, 68 sera samples were found to be positive for FMD.

3.6.4 Rose Bengal agglutination test for Salmonellosis

Rose bengal agglutination test was used to detect antibodies against *Salmonella* antigens H4,12; O4,12 and O9,1. Results revealed that 4 free ranging sambars were seropositive for antibodies against H4, 12; 4 against O4,12 and 3 against O9, 12. Three animals were positive for more than one antigen. In all, 6 animals had the titre beyond normal range for one or more of the antibodies against these antigens.

Using tube agglutination test, sera were tested for the presence of antibodies against *Salmonella* Typhimurium, Enteritidis, Dublin and Abortus ovis. The sera from buffalo and cheetal were not found to possess antibodies against above antigens, however 2 samples from Sambars and nine from goats were found to possess antibody titres beyond normal values.

3.6.5. Elisa for Babesiosis

The sera were tested using ELISA for *Babesia bigemina*. The test for sambar was conducted employing the anti-bovine conjugate while the same for cheetal serum was done using the anti-goat conjugate. The results are summarized in table 11.

Table 11. Results of Elisa for Babesiosis

Sl. No.	Animal	No. examined	Positive
1.	Sambar	32	2
2.	Cheetal	01	Nil
3.	Goat	55	4
4.	Buffalo	74	14

Results of serology on various serum samples collected at Sariska Tiger Reserve are listed in table 12:

Table 12. Results of serology

Sambar Deer

Sample No.	BTV	Salmonellosis H4,12 O4,12 O9,12	FMDV	Leptospirosis	Remarks
S 1	-	-	-	+	
S2	-	-	-	-	
S3	-	-	-	+	
S4	-	-	-	-	
S5	-	-	-	-	
S6	-	++ ++ ++	-	-	
S7	-	± - -	-	-	
S8	-	-	-	-	
S9	-	-	-	-	
S10	-	-	-	-	
S11	-	-	-	-	
S12	-	-	-	-	
S13	-	-	-	-	
S14	-	- ++ -	-	-	
S15	-	-	-	+	
S16	-	-	-	-	
S17	-	-	-	-	
S18	-	-	-	-	
S19	-	-	-	-	
S20	-	-	-	-	
S21	-	-	-	-	
S22	-	-	-	-	
S23	-	- + +++	-	-	
S24	+	-	-	-	Precipitation line in BTV AGPT towards serum well
S25	-	+ ++ ++	-	-	
S26	-	-	-	-	
S27	+	-	-	-	Line of identity with the control in BTV AGPT
S28	-	-	-	-	
S29	-	-	-	-	
S30	-	-	-	-	
S31	-	++ - -	-	-	
S32	-	-	-	-	
C1	-	+ - ++	+	-	

Blue tongue positive - 2, Salmonellosis positive – 5, Foot & Mouth Disease positive – 1 cheetal, Leptospirosis positive – 3

Goats

Sample No.	BTV AGPT	Salmonellosis H4,12 O4,12 O9,12	FMDV	Leptospirosis	Remarks
G1	-	Not tested	-	-	
G2	-		-	-	
G3	-		-	-	
G4	-		-	-	
G5	+		-	-	
G6	-		-	-	
G7	+		-	-	
G8	+		-	-	
G9	-		+	-	
G10	+		+	-	
G11	-		-	-	
G12	+		-	+	
G13	+		-	-	
G14	+		-	-	
G15	+		-	-	
G16	+		-	+	
G17	-		-	-	
G18	-		-	-	
G19	+		-	-	
G20	+		-	-	
G21	+		-	-	
G22	+		+	+	
G23	+		-	-	
G24	+		-	-	
G25	-		-	-	
G26	-		-	-	
G27	-		-	-	
G28	-		-	-	
G29	+		-	-	
G30	+		-	-	

G31	-	-	-
G32	-	-	-
G33	+	-	+
G34	+	+	-
G35	-	-	-
G36	-	+	-
G37	-	-	-
G38	-	+	-
G39	-	-	-
G40	-	-	-
G41	-	-	-
G42	-	-	-
G43	-	-	-
G44	-	+	+
G45	-	-	+
G46	-	-	+
G47	-	+	-
G48	-	-	-
G49	-	+	-
G50	+	+	-
G51	-	+	-
G52	-	+	-
G53	-	+	-
G54	-	+	-
G55	-	+	-

Blue tongue positive – 20, Foot & Mouth Disease positive – 15, Leptospirosis positive – 7

Cattle and Buffalo

Sample No.	BTV AGPT	Salmonellosis H4,12 O4,12 O9,12	FMDV	Leptospirosis	Remarks
D1	-	Not tested	-	-	
D2	-		-	-	
D3	-		-	-	
D4	-		-	-	
D5	-		-	-	
D6	-		-	-	
D7	-		-	-	
D8	-		-	-	
D9	-		-	-	
D10	-		-	-	
D11	-		-	-	
D12	-		-	-	
D13	-		-	-	
D14	-		-	-	
D15	-		-	-	
D16	-		-	-	
D17	-		-	-	
D18	-		+	-	
D19	+		-	-	
D20	-		-	-	
D21	-		-	-	
D22	-		-	-	
D23	-		-	-	
D24	-		-	+	
D25	-		-	-	
D26	-		-	-	
D27	-		-	-	
D28	-		-	-	
D29	-		-	-	
D30	-		-	-	

D31	-	-	-
D32	-	-	-
D33	-	-	-
D34	-	+	-
D35	-	+	-
D36	-	-	-
D37	+	-	-
D38	-	-	-
D39	-	+	-
D40	+	+	-
D41	+	-	-
D42	-	+	-
D43	-	+	-
D44	-	+	-
D45	-	+	-
D46	-	+	-
D47	-	+	-
D48	-	+	-
D49	-	+	-
D50	+	+	-
D51	-	+	-
D52	-	-+	-
D53	-	+	-
D54	-	+	-
D55	-	+	-
D56	-	+	-
D57	-	+	-
D58	-	+	-
D59	-	+	-
D60	-	+	-
D61	+	+	-
D62	+	+	-
D63	+	+	-

D64	-	+	-
D65	-	+	-
D66	+	+	-
D67	-	+	-
D68	-	+	-
D69	-	+	-
D70	+	+	-
D71	+	+	-
D72	-		-
D73	+	+	-
D74	+	+	-
D75	-	+	-
D76	-	+	-
D77	-	+	-
D78	-	+	-
D79	-	+	-
D80	-	+	-
D81	-	+	-
D82	-	+	-
D83	-	+	-
D84	-	+	-
D85	-	+	-
D86	-	-	-
D87	-	+	-
D88	-	-	-
D89	-	+	-
D90	-	+	-

Blue tongue positive – 13, Foot & Mouth Disease positive – 51, Leptospirosis positive -1

3.7. Microbiological examination

3.7.1 Sambar nasal swab samples for isolation of bacteria

Out of 8 samples of nasal swab of sambar deer from Sariska Tiger Reserve, Rajasthan, only one was found positive for *Pastuerella haemolytica*. All the samples were quite old, hence isolation frequency was low.

3.7.2 Sambar rectal swab samples for isolation of bacteria

A total of 11 isolates of *E. coli* were isolated from 32 rectal swabs from apparently healthy free-ranging sambars. Of these, 2 cultures namely S-1 and S-2 were missed during processing after identification as *E. coli*. Thus 9 isolates were left for further studies and 9 isolations were reported in all further references. These 9 isolates belonged to 5 different serogroups. The detailed antibiogram was presented in Table 13, sugar fermetation and motility results were presented in Table 14 and summary of the results on pathogenic attributes is presented in Table 15.

Table 13. Antibiogram of *Escherichia coli* isolates from rectal swabs of sambar deer

(Figures indicate the size of inhibitory zone of antimicrobial agents in mm)

	Antimicrobials*										
Isolate	**Na**	**Co**	**Cf**	**Nx**	**C**	**K**	**O**	**S**	**Cp**	**N**	**F**
S-6	20(S)	24(S)	30(S)	24(S)	22(S)	15(I)	17(I)	13(I)	14(R)	12(R)	20(S)
S-7	22(S)	25(S)	24(S)	24(S)	24(S)	16(I)	17(I)	16(S)	16(I)	12(R)	14(R)
S-8	19(S)	24(S)	26(S)	18(S)	26(S)	18(S)	20(S)	18(S)	11(R)	17(I)	18(S)
S-9	20(S)	22(S)	25(S)	22(S)	28(S)	18(S)	18(I)	17(S)	15(I)	13(I)	18(S)
S-10	20(S)	28(S)	30(S)	24(S)	20(S)	20(S)	18(I)	17(S)	17(I)	16(I)	18(S)
S-11	20(S)	26(S)	30(S)	26(S)	24(S)	20(S)	18(I)	20(S)	15(I)	16(I)	19(S)
S-13	20(S)	20(S)	20(S)	20(S)	26(S)	17(I)	18(I)	17(S)	11(R)	16(I)	16(I)
S-31	20(S)	20(S)	20(S)	18(S)	24(S)	13(R)	14(R)	13(I)	10(R)	11(R)	14(R)
S-32	20(S)	22(S)	21(S)	24(S)	25(S)	16(I)	15(I)	17(S)	12(R)	14(I)	18(S)

Key : S : Sensitive; I : Intermediate; R : Resistant

*Na : Nalidixic acid (30 µg); Co : Co-trimoxazole (25 µg); Cf : Ceprofloxacin (10 µg);
Nx : Norfloxacin (10 µg); C : Chloramphenicol (30 µg); K : Kanamycin (30 µg);
O : Oxytetracyclin (30 µg); S : Streptomycin (10 µg); Cp : Cephalexin (30 µg);
N : Neomycin (30 µg); F : Furazolidone (30 µg)

Table 14. Biochemical characteristics of *Escherichia coli* isolates from rectal swabs of sambar deer

Test	Isolate								
	S-6	S-7	S-8	S-9	S-10	S-11	S-13	S-31	S-32
TSI	-	-	-	-	-	-	-	-	-
Simmon's Citrate	-	-	-	-	-	-	-	-	-
Urease	-	-	-	-	-	-	-	-	-
Indole	+	+	+	+	+	+	+	+	+
MR	+	+	+	+	+	+	+	+	+
VP	-	-	-	-	-	-	-	-	-
Lecithinase	-	-	-	-	-	-	-	-	-
H_2S Production	-	-	-	-	-	-	-	-	-
Sugar fermentation									
Lactose	+	+	+	+	+	+	+	+	+
Sucrose	+	+(D)	+	-	-	-	+	+(D)	+(D)
Glucose	+	+	+	+	+	+	+	+	+
Malonate	-	-	-	-	-	-	-	-	-
Motility									
	+	+	+	+	+	+	+	+	+

Table 15. Pathogenic Attributes of *Escherichia coli* Serotypes from free-ranging sambar deer

Sample No.	Serogroup	Sugar fermentation* (Sucrose)	Antibiotic resistant to	Congo red dye adsorption 18h	Congo red dye adsorption 36h	Klebobiocin sensitivity	MSC E	Surface anionicity	Sephdex binding	Hydro-phobicity	Cyto-toxicity	Patho-genicity Morbidity (Mortality) %
S6	O:6	+	Cephalexin, Neomycin	-	+	-	00	7.06(±1.48)	5.50	18.76	+++	60(35)
S7	O:38	D	Neomycin, Furazolidone	-	+	-	00	20.85(±6.90)	6.05	18.00	+++	66(00)
S8	O:96	+	Cephalexin	-	+	-	00	18.30(±4.50)	2.16	18.30	+++	33(33)
S9	O:66	-	-	-	+	+	2.00	18.91(±1.17)	3.38	21.18	+++	100(66)
S10	O:11	-	-	+	+	+	1.25	13.58(±3.76)	3.10	13.50	+++	100(00)
S11	O:38	-	-	+	+	-	0.50	27.62(±9.15)	3.15	19.58	-	00(00)
S13	O:96	+	Cephalexin	+	+	-	1.25	23.92(±6.97)	4.60	23.92	+++	00(00)
S31	O:96	D	Kanamycin, Oxytetracyclin Cephalexin, Neomycin, Furazolidone	-	+	-	0.25	15.18(±6.69)	2.26	28.54	+++	66(33)
S32	O:96	D	Cephalexin	+	+	-	>2.00	18.58(±4.96)	3.57	18.58	-	00(00)

* : All the isolates fermented lactose and glucose but sucrose fermentation varied

E : Molar salt concentration required for salt aggregation of test strain

: negative results ; D : delayed fermentation

CHAPTER 4
DISCUSSION

4.1. Haematology and biochemistry

Blood examination is a good tool for the evaluation of animal health. It provides the information regarding functional state of the different systems and organs of the body.

The primary function of the erythrocyte is to serve as a carrier of haemoglobin, which carries O_2 and CO_2. Erythrocyte contributes to blood volume by means of its mass. Reduction in total erythrocyte count (TEC), packed cell volume (PCV) and haemoglobin concentration indicates anaemic state of the animal. The anaemia may be haemolytic (toxicity or poisoning), haemorrhagic (parasitic, bacterial, viral or trauma) and nutritional (protein or iron deficiency) type.

Total and differential leucocyte counts indicate systemic response of the body against the bacterial, viral, fungal or parasitic diseases. It also suggests the period of sickness and prognosis of the individual against the infectious diseases.

Increase in erythrocyte sedimentation rate is commonly seen when body defence mechanism is working against the chronic inflammation. The increased ESR is generally seen in chronic wasting diseases like TB and John's diseases.

Examination of the blood smear is very important as it provides information on health status of the animals by examining the RBC morphology, response of body defence mechanism by evaluating differential leucocyte count (DLC) in diseased conditions and also we can detect some of the blood protozoan infections by examining blood smears, like trypanosomiasis, theilariasis, babesiosis and toxoplasmosis, etc. Significance of blood parameters studied is summarised in the table 16 & 17.

Table 16. Significance of haematological parameters

1.	Total erythrocyte count (TEC)	Increase is seen in polycythemia, haemoconcentration as a result of diarrhoea or continuous vomiting. The decrease is suggestive of anaemia especially chronic, hemolytic or toxic. The results should be interpreted in conjunction with haemoglobin concentration and PCV.
2.	Haemoglobin Concentration	The decrease of haemoglobin concentrations are usually associated with anaemia. Cells in such conditions will be hypochronic or microcytic or both in some instances. Thus for the final diagnosis, the quality and nature of the RBCs should be critically examined in stained smears.
3.	Packed Cell Volume (PCV)	While estimating the PCV, one can judge various conditions by examining the erythrocytes, buffy coat or plasma. PCV values rise in hemoconcentration while a decrease is associated with anaemia. Leucocytosis or leucopenia can be qualitatively judged by seening the buffy coat in Wintrobe method. Colour of plasma is reflectiv eof jaundice, hemolytic anaemia or lipemia. However, dehydrated animals with low PCV will give approximately normal values.
4.	Total Leucocyte count (TLC)	A pathological increase in LTC is associated with viral infection while physiological increase is observed due to exercise, stress, fear, estrus, pregnancy, digestion and other conditions related with corticosteroid release in the body. A high value is seen in young calf and dog while low in young pig.
5.	Erythrocyte Sedimentation Rate (ESR)	Erythrocyte fall in plasma due to gravitatinal force and this sedimentation rate is increased in inflammatory conditions and bacterial infections.
6.	Differential Leucocyte Count (DLC)	Alongwith the counting of various types of WBCs in the blood smear for diagnostic aid in different disease conditions, abnormalities in the morphology of RBCs or WBCs like variation in colour, size and shape and presence of inclusions suggest for many conditions of anaemia and infection. Acute diseases lead to leucocytosis and neutrophilia while in viral infection, leucopenia with lymphocytosis is seen. In allergy, eosinophilia is noticed.

Table 17. Significance of various biochemical parameters

Sl No.	**Parameters**	**Significance**
1.	SGOT (Serum Glutamate Oxaloacetate Transaminase) (AST)	Organs rich in GOT are heart, liver and skeletal muscles. When any of these organsare damaged, the serum GOT level rises in proportion to the severity of the damage. In hepatitis, GOT peaks usually between 7-12 days and may increase upto 100 times. Increased levels are also found in pancreatitis, trauma of skeletal muscle, renal necrosis and cerebral necrosis.
2.	SGPT (Serum	Elevation of serum GPT activity is found in liver and kidney diseases

	Glutamine Pyruvate Transaminase) (ALT)	such as infectious or toxic hepatitis and cirrhosis. A modeate increase is also found in obstructive jaundice, metastatic carcinoma, hepatic congestion and myoardial infraction.
3.	Urea	Urea is the main end-product of protein metabolism. Liver is the site of urea synthesis and urea is excreted through kidneys. Increase of serum urea concentrations are associated with dehydration, shocks, fevers, acute glomerulonephritis and urine retention. Low serum urea levels in clincial diseases such as severe liver damage due to viral hepatitis are rare.
4.	Glucose	Reflects the energy level of the animal at a given time, thus reflects nutritional status of the animal and the functions of throid and parathyroid. Increased levels are generally indicative of diabetes.
5.	Sodium and Potassium	The electrolyte level in the body reflects their balance and may be of diagnostic value in cases of dehydration, renal disturbances etc.
6.	Chloride	Determination of the chloride concentration in serum has an important diagnostic value in reviewing the maintenance of osmotic pressure, water distribution and pH of the body. Hypochloremia is observed in salt-losing nephritis like chronic pyelonephritis and metabollic acidosis like diabetic ketoacedosis or renal failure. Hyperchloremia is observe dwith dehydration, renal tubular acidosis, acute renal failure, metabollic acidosis associated with prolonger diarrhoea and respiratory alkalosis.
7.	Calcium	Elevated calcium values are asosicated with hyperparathyroidism, multiple myeloma, neoplasias of bone an dparathyroid and conditions of rapid demineralisation of bone. Lowered calcium levels are associated with hypoparathyroidism, tetany and ocasionally with nephrosis and pancreatitis. Severe nephritis and uremia may cause either elevated or lowered calcium values.
8.	Total protein	Serum protein concentration decrease in malnutrition, albuminuria, liver diseases whereas increase or protein occurs in multiple myeloma, chronic infections and dehydration. Change in serum total protein concentration can be due to change in one or many fractions of the protein and hence, the total protein determination should be followed by albumin and A:G ratio determination.
9.	Albumin, Globulin and A:G ratio	Hypoalbuminuria is found in any liver impairment, nephrosis, certain chronic diseases, malnutriton, severe haemorrhage and pregnancy. Lowering of serum albumin usually results in lowering of A:G ratio. Elevated serum albumin levels apart from idnicating dehydration are generally of little significance. Globulines are, however, important as a fraction of these proteins i.e. immunoglobulins play important role in the humoral defence mechanism.
10.	CPK (Creatinine Phospho Kinase)	Present primarily in skeletal muscles, myocardium and brain. Following myocardial infraction, CPK activity begins to rise within 4 hours and peak values are reached in 18-30 hours which return to normal in 3-4 days. Elevated CPK activity is also found in muscular dystrophy, hypothyroidism, pulmonary and cerebrovascular diseases.

11.	DLH (Lactose Dehydrogenase)	Increased activity is observed in myocardial infraction, pernicious anaemia, hepatitis, malignancy, muscle dystrophy and pulmonary embolism. The ratio between enzyme activitie sin pleural fluid and serum is usually less than 0.6 in transudates and more than 0.6 in exudates in case of tuberculosis. Since R.B.Cs. are a potential source of LDH, the specimen must be totally free from hemolysis.
12.	Inorganic Phosphorus	Serum inorganic phosphorus levels have proven to be of value in evaluating the vitamin D deficiency, malabsorption syndromes, hyperinsulinism, steatorrhoea, primary hypophosphataemia, osteomalacia, rickets, hyperparathyroidism and diabetes mellitus. In diabetes mellitus, the serum inorganic phosphorus concentration may become elevated due to renal disease, a complication of diabetes. In enal diseases, inorgnic phosphorus concentration reaches an elevated state late in the course of illness and this elevation may have the same prognostic implications as does elevated serum creatinine. Elevated serum inorganic phosphorus levels can also occur during the healing of bone fractures, ture and pseudohyperparathyroidism, vitamin D intoxication and dehydration and in some cases of osteolytic metastatic tumour of the bone.
13.	Alkaline phosphatase	The increase of serum alkaline phosphatase activity is related to bone regeneration an is increased in rickets and osteomalacia. The activity is also found to be increased in post hepatic jaundice as well as infective or toxic hepatitis. A physiological increase is observed in pregnancy. Many drugs cause increase in alkaline phosphatase activity e.g. endrogens, anabolic steroids, estrogens, sulphonmides, antibiotics, anticoagulants, diuretics, some immunosuppressants and anticonvulsants etc.
14.	Creatinine	Increased serum creatinine is a very good index of kedney failur or muscular dystrophy. From the urine and serum creatinine values, the creatinine clearance is calculated which gives an indication of kidney function.
15.	Total Bilirubin	Bilirubin determinations in serum is used for the diagnosis, differentiation and follow up of jaundice. Elevation in serum unconjugated bilirubin levels occur in hemolytic jaundice due to excessive hemolysis. The conjugated bilirubin is predominantly increased in obstructive jaundice due to regurgitation while hepatic jaundice is associated with increase in both conjugated and unconjugated bilirubin in serum. Generally, along with serum bilirubin determination, other tests such as SGPT, alkaline phosphatase, etc. are also carried out to assess liver function.
16.	Cholesterol	Cholesterol determination is a good index of the risk of coronary heart disease (CHD). CHD risk factor can be calcualted using total lipid profile. The risk factor gives a most accurate and definite assessment of heart disease risk.

17.	Uric Acid	Uric acid is the principal end product of nucleic acids and purine metabolism. Uric acid level is little affected by variation in the purine content of the diet and represents a steady state between endogenous synthesis and urine excretion. Raised uric acid levels in serum may be found in patients of gout, leukaemia, myeloproliferative diseases and often in pernicious anaemis. Treatment with adrenocorticotrophic hormone or corticosteroids can cause a rise in uric acid. In renal failure, or when there is any urinary obstruction, uric acid is retained and its level in serum rises. In renal tubular syndromes, uric acid levels may be decreased.
18.	γ-GT (Gamma Glutamyl Transferase)	The enzyme γ-GT is seen mainly in the liver, followed by that in the kidneys and in the pancreas. Its levels are usually increased in hepatitis, cirrhosis, cholangitis, choleocystitis and hepatic carcinoma. It forms an important parameter in assessing liver functions. Since serum γ-GT is not elevated in any form of bne disorder, its assay has been suggested as a valuable diganostic aid in differentiating bone and liver disease in conjunction with alkaline phsphatase determination.

4.2. Serological investigations

Serological anslysis is very helpful in the detection of the infectious diseases. These examinations are based on antigen-antibody reactions. The adequate level of antibody is responsible for the immunity, which provides protection to the animal against future infections. Antibodies are specific against a particular infection. Antibody titre gives an idea about the present level of infection or previous exposure to the infection. These tests requires sophisticated instruments, hence can be done only in well established laboratories.

The antibody titre in the serum gives an idea about the present infection or previous exposure to infection and indicates the prevalence of a disease in that geographical area. It is also possible to forecast the occurrence of the disease in future based on long term serological data supported with other information on meteorological parameters, geography, and animals in question in a particular season. Serum immunoglobulin levels are dependent on a variety of developmental, genetic and environmental factors including ethnic background, age, sex, geographical factors, history of allergies and recurrent infections. *i.e.* endemic infestations with parasites result in elevated IgE levels.

4.2.1. Salmonellosis

Diarrhoeal syndrome in humans is extremely common in tropical and developing countries and significantly contributes to death **(Barua, 1981).** Among animals, acute undifferentiated diarrhoea of newborn has been found to be a major cause of economic losses. *Salmonella* has been known to be associated with gastroenteritis in man and animals **(Anderson *et al.*, 1960).** It is recognized to be one of the important causes of diarrhoea in India and abroad **(Baker, 1975; Fule and Kaundinya, 1985; Ling *et al.*, 1987).** Among all the causes of bacterial food poisoning, *Salmonella* has gained importance because of its increasing incidence. Furthermore, salmonellosis occurs with regularity in the population and is considered to be the most important zoonosis even in developed countries. The organism is widely distributed in nature with a huge animal reservoir, infecting man and animals alike. Salmonellae have been isolated from all wild and domesticated animals, birds and reptiles, confined fur-bearing species, laboratory primates and rodents and many species of wild rodents **(Williams and Hobbs, 1975).**

In India, prevalence of *Salmonella* in animals has been reported from almost all parts of the country. Different serotypes of *Salmonella* have been isolated from domestic animals including cattle, buffalo, sheep, goat, pig, dog and poultry, wildlife such as turtle, lizard, python, jackal, bat, snake, mongoose, field rats, turtle, toad and free flying birds including parrot, crow, house sparrow, swallow myna (Sharma and Thapliyal, 1995). A vast array of *Salmonella* serotypes have been detected in foods of animal origins (Sharma *et al.,* 1995). As a zoonosis, the disease has assumed increasing importance in recent years because of the frequent occurrence of human salmonellosis, with animal salmonellosis as the principal reservoir. Transmission to man occurs via contaminated drinking water, milk or meat.

Species specific agglutinins are detectable in the serum of infected animals and have uses as diagnostic aids. However, they do not appear in the serum until about 2 weeks after infection and many animals carry moderate titres against *Salmonella* sp. although they are not infected, so that a positive serological test cannot be depended on to identify individual infected animals. That is why a titre of 1 in 400 and above was considered specific to declare a case seropositive in the present study.

4.2.2. Leptospirosis

Leptospirosis is a bacterial disease of domestic animals, wildlife and man caused by members of the genus *Leptospira*. The serovars most commonly coming across are pomona, icterohaemorrhagiae, australis, autumnalis, hebdomadis, hardjo, canicola, pyrogenes and grippotyphosa among others. In domestic ruminants, leptospirosis produces a short term fever, loss of appetite, anaemia, blood stained urine and pneumonia. Pregnant animals may abort 1-4 weeks after exposure due to *Leptospira* invading the foetus. A number of serological surveys for leptospirosis in wildlife have been conducted in North America which indicate a seropositivity among wildlife, although failure to isolate the organism from exposed animals is sufficient reason to view all serological test results with caution. In India, the status of infection is summarised in the following table:

Distribution of Leptospira Serovars in India (based on isolation and serology)

State	Animal Species	Serovars
High Prevalence		
Tamil Nadu, Kerala, Andaman	Cattle, Buff, sheep, goats, pig	pomona, icterohaemorrhagiae, australis, autumnalis, hebdomadis
Moderate Prevalence		
Maharashtra, U.P., M.P., Gujarat, Karnataka	cattle, buff, goats, sheep, pigs, dogs, horse	pomona, Hardjo, andamana, canicola icterohaemorrhagiae, pyrogenes, Javanica
Rarely reported		
Punjab, J&K, Rajasthan, North-Eastern Hills, H.P.	Cattle, Sheep	icterohaemorrhagiea pyrogenes, canicola

Control of Leptospirosis can be achieved by the following methods:

1. Control of Infection

- Isolation of diseased animals
- Hygienic measures
- Chemotherapy- Dihydrostreptomycin, ciprofloxacin

2. Prevention by vaccination: 10ml of killed vaccine, I.M., twice at 4 weeks of interval. Repeated at 6 months (which is quite not possible in free-ranging wildlife but none the less, can be easily adopted in domestic animals coming at the interface to reduce the reservoir of the infection in domestic animals).

3. Elimination of source of Infection

- Disinfection of animal premises, field areas, clothing etc. (Sod. hypochlorite)
- Disinfection of ponds ($KMNO_4$)
- Rodent control (Zinc phosphide)

4. Education of professional groups (farmers, veterinarians, forest officials, public health workers etc.)

Leptospirosis, a bacterial disease of domestic animals, wildlife and man, is caused by members of genus *Leptospira*. There are about 150 distinct *Leptospira* serovars, all pathogenic serovars placed under species *L.interrogans*. The disease has been recorded in animals and man throughout the major continents of the world. The disease is widely prevalent in India and has been recorded from cattle, buffalo, horse, pig, sheep, goat and dog. Among wild mammals, the seroprevalence of the disease has been reported in captive elephants (2/15), tiger (1/1), chinkara (1/1), blackbuck (1/3) and free-ranging tiger(1/1), lion (1/5), sambar (1/1), nilgai (1/2) and swamp deer (2/4). Various serovars reported have been *valbuzzi, pyrogenes, semaranga, autumnalis, pomona* and *patoc* from wild stock. However, no systematic study has been conducted among wild animals at wildlife-domestic interface. In an attempt in this direction, a total of 178 sera samples, collected from domestic cattle (4), buffaloes (86) and goats (55) as well as free-ranging sambars (32) and cheetal (1) following immobilization were tested using microscopic agglutination test to study the sero-prevalence of leptospirosis in the present study. Results indicated a positivity among 7 goats (12.73%), 1 buffalo (1.16%) and 3 sambars (9.37%) for various *Leptospira* serovars. Common serovars detected among wild and domestic animals were *pyrogenes* or *tarassovi*; other serovars being *patoc* and *ballum*.

4.2.3. Blue tongue

Blue tongue is a disease of domestic sheep, cattle, goats, and wild ruminants caused by a virus of the *Orbivirus* genus. The name bluetongue is derived from a clinical sign

occasionally seen in domestic sheep in which the oral mucosa and tongue appear dark blue, due to cyanosis. The disease in domestic sheep is characterized by fever, depression, nasal discharge, swelling of mucous membranes, ulceration of the tongue and foot lesions producing lameness. Death may occur about 6 days after first sign of illness appear. In flocks, where the disease has not previously occurred, death rates may reach 50%. Sheep that recover undergo a long convalescence and loss of physical condition. The pregnant ewes that recover may give birth to lambs with severe brain damage due to effect of the virus upon the foetus. The clinical disease in cattle and goats is generally much milder with little or no mortality. The virus is transmitted by a small biting gnat, *Culicoides* sp. The disease trend in livestock varies with the prevalence of this gnat, which peaks in warmer and rainy season.

In India, the virus is widely prevalent in domestic and wild ruminants (Prasad *et al.*, 1992). In last two years, several outbreaks of bluetongue have occurred resulting in high mortality in the states of Tamilnadu, Karnataka, Andhra Pradesh and Maharashtra.Blue tongue is list A disease of OIE thereby restriction is imposed on movement of animals and their germplasm to the countries free of BTV. Multiplicity of virus serotypes and diversity of *Culicoides* vector makes it difficult to control this disease.

The serological survey was carried out at Sariska Tiger Reserve to determine whether there is transmission of BTV from domestic to wildlife or *vice versa*. Two sambars and several goats and buffaloes serum samples were positive for BTV antibodies. Further investigations are required to understand the dynamics of BTV infection in wild ruminants. Since BTV is endemic in the country, extensive studies are required on regular basis.

BTV belongs to *Orbivirus* genus of the family *Reoviridae*. It is transmitted by *Culicoides* midges to domestic and wild ruminants. Although all the ruminants are susceptible to BTV infection, sheep is the most susceptible species for the clinical bluetongue disease. Though cattle, buffalo, goat and wild ruminants are susceptible to BTV infection, they rarely develop clinical disease. Nevertheless, these ruminant species play an important role in the epizootiology of the disease and serve as reservoirs of BTV infection. There are 24 serotypes of BTV known all over the world (Knudson and Shope, 1985). BTV infection is widely prevalent in domestic ruminants in India (Prasad *et al.*, 1992). Wild animals have been reported to be susceptible to BTV infection in several countries (Jessup *et al.*, 1984 and

Alexander *et al.*, 1994). However, there is complete lack of information regarding prevalence of BTV in wild ruminants in India. Sariska Tiger Reserve is one such wildlife reserve which is surrounded by human settlements with large domestic animal population which freely enters into the reserve for grazing and come in contact with wild animals.

Recently prevalence of BTV group specific antibodies have been reported in elephants (*Elephas maximus*), blackbucks (*Antelope cervicapra*) and spotted deer (*Axis axis*) in Tamil Nadu (Narayana and Manickam, 1997). In an extensive survey of BTV infection in domestic livestock in Rajasthan, prevalence of BTV antibodies has been reported from almost all the regions of the state including Jaipur, Sikar and Bharatpur districts which surround Alwar district where Sariska Tiger Reserve is located (Srivastava *et al.*, 1995).

Domestic animals freely enter into the reserve for grazing and come in contact with wild animals. Therefore, there is possibility of transmission of BTV from domestic to wild and from wild to domestic animals.

The preliminary reports from India indicate that BTV infection appears to be prevalent in several species of Indian wild ruminants (Narayana and Manickam, 1997). However, the role of wildlife in occurrence of BTV infection in domestic ruminants remains to be investigated. Further studies are necessary to identify the insect vector, related Orbiviruses and seasonallity of BTV infection in the wildlife reserves.

4.2.4 Foot and Mouth Disease (FMD)

Foot and mouth disease is an extremely contagious, acute disease of all cloven-footed animals, caused by a virus and characterized by fever and vesicular eruption in the mouth and on the feet. FMD is enzootic in Africa, Europe, Asia, Japan and South America. Major strains of the causative enterovirus are A, O and C alongwith Asia-1 form Asia; SAT1, SAT2 and SAT3 are reported from Africa. Of these strains, O appears to be the most common, followed by Asia 1, A and type C being the least. There is no cross immunity between strains and substrains. Losses due to the disease occur in many ways although loss of production, the expense of eradication and the interference with movement of livestock and meat between the countries are most important economic losses. Many wild ruminants are susceptible and may provide reservoirs of infection for domestic animals or *vice versa*.

In India, the incidence is more in cattle and buffaloes followed by pigs, sheep and goats among the domestic animals. The disease is more severe in exotic and crossbred animals than in indigenous livestock. The disease has also been reported in wild ungulates like bison, nilgai, deer, sambar, wild buffalo and elephant. The virus is endemic in India and outbreaks occur throughout the year in all the regions of the country.

Inactivated vaccine against FMD is being produced in India which incorporates all the four prevalent types of the virus. Despite reported vaccination, outbreaks of FMDV continue to occur throughout the year and the immunity produced by the existing vaccine is short lived and protects animals only for 3 months after vaccination. This indicates the limitation of BHK-21 cell culture produced vaccine which is currently in use. Therefore, alternative vaccine needs be explored to enhance the potency of vaccination.

4.2.5 Babesiosis

Babesiosis is a tick-borne infectious haemoprotozoan disease of cattle, buffalo, horse, sheep, goat, dog, pig, wild animals and man caused by *Babesia* sp. The disease is characterized by pyrexia, haemolytic anaemia, haemoglobinaemia, haemoglobinuria, jaundice and death. The native animals in enzootic areas acquire immunity, through inapparent infection and become carriers. Under stress (due to malnutrition, parturition or inclement weather), they may act as source of infection to newly introduced suceptible hosts. Examination of stained/unstained blood smears is a rapid method of detection of the infection, however, a number of serological tests are also available for detecting the exposure of animal to the infection. Treatment of the sick animals is reported to be successful although supportive therapy is a must in acute infections.

The disease is world wide in distribution. The disease has got a serious economic impact due to high mortality rates, decreased production and lowered working eficiency. In India, there has been a great deal of information available on babesiosis in cattle but there are sporadic reports of the disease in wildlife. There is high percentage of sero reactors among cattle of different states of the country. Among wild animals, the disease has been reported from artiodactylids (deer, bison, mithun), carnivores (jackal, wild cats, leopard, tiger, mongoose, lion), primates, elephants, rodents and man.

4.3 Microbiological Studies

Sambar rectal swab samples for isolation of bacteria

Escherichia coli has often been found to be associated with a variety of ailments, including gastro-enteritis, urinary tract infections, wound infections and septicaemia (**Sojka, 1971; Senior *et al.*, 1992; Kapur *et al.*, 1992**). This is particularly true in compromised animals under the stressful conditions like under-nourishment, unhygienic and inadequate water supply, concurrent infections, parasitic infestations, extremes of temperature, overcrowding *etc.* Although *E.coli* strains belonging to serogroups O:157, O:26 and O:128 are considered pathogenic (**O'Brian, *et al.*, 1982**), their pathogenicity depends largely on their pathogenic attributes. Isolation of *E.coli* from apparantly healthy as well as diseased wild animals has been reported (**Arora, 1994**). However, information on pathogenic attributes of the isolates is lacking.

The results of the present investigation revealed that most common serogroup was O:96 (4/9) followed by O:38 (2/9) while one each belonged to serogroups O:6, O:11 and O:66. All the isolates fermented lactose and glucose but sucrose fermentation varied as given in Table . Five isolates were found resistant to cephalexin while 3 were resistant to neomycin. One isolate showed multiple resistance against five antibiotics.

Thus all isolates were resistant to one or more antibiotics. However, no exposure to antibiotics is expected in free-ranging sambars. It indicates that the infection might have originated from the domestic animals coming in contact with these wild herbivores during grazing or at common water source etc. The findings, therefore, suggest cross infection of various diseases/ disease agents among wild and domestic animals.

Cytotoxicity of cell-free culture filtrates on MDBK cells indicated that 7 of the 9 isolates were cytotoxigenic causing necrosis and detachement of cells within 24 h.

The pathogenicity tested by intra-peritoneal administration of cultures in mice indicated that 6 out of 9 isolates were pathogenic to mice. On post-mortem examination, congestion in lungs, sub-cutaneous tissues and intestine (enteritis) were observed. The result of cytotoxicity and pathogenicity assays were parallel except that S13 (O:96) was cytotoxigenic but not pathogenic to mice. The magnitude of pathogenicity of different isolates in terms of mortality and morbidity varied considerably.

The results of mice mortality and MDBK cytotoxicity assay indicated the pathogenic nature of isolates, though all the swabs were collected from apparantly healthy animals showing no signs of any disease. This is significant as wild animals harbouring pathogenic organisms can serve as a source of infection to domestic animals.

Congo red dye adsorption was observed in 3 isolates, namely S10 (O:11), S11 (O:38) and S13 (O:96) at 18 h, while all isolates adsorbed the dye in 36 h. Two isolates, namely S9 (O:66) and S10 (O:11) were sensitive to klebobiocin while rest were resistant to it. Molar salt concentration was maximum for isolate S32 (>2.00) indicating highest surface hydrophobicity while three isolates (S6, S7, S8) showed no salt aggregation. Rest of the isolates showed different MSC for salt aggregation (0.5 to 2.00). Surface anionicity of S11 (O:38) was the highest followed by S13 (O:96) and S7 (O:38). S6 (O:6) strain showed the lowest anionicity. The sephadex binding, however, was the highest in S7 (O:38) followed by S6 (O:6). Of all the isolates, only S31 (O:96) showed intermediate adherence to n-hexadecane, while others were found negative for adherence (less than 25%).

The ability to bind congo red in agar medium has been proposed as a marker for the invasive ability of pathogenic *Escherichia coli* (**Payne and Finkelstein, 1977**), *Shigella* sp. (**Chambers *et al.*, 1985**) and other enteropathogens. However, no direct correlation between CRDA and mice pathogenicity or MDBK cell cytotoxicity was determined in the present study. The results also indicated lack of relationship between the pathogenicity of isolates and their serogroups because organisms belonging to certain serogroup generally considered to be pathogenic were found to be non-pathogenic/ non-cytotoxigenic and *vice-versa*. Although an association between surface hydrophobicity and pathogenicity of bacteria has been reported (**Pascual *et al.*, 1986**), on the basis of results of hexadecane adherence and salt aggregation tests, such a correlation could not be established in the present study. Similar findings were also reported by **Greene *et al.* (1992)** for *Staphylococci*. These attributes, however, may possibly be used as epidemiological tools for typing the organisms as envisaged by **Greene *et al.* (1992)**.

4.4 Post mortem examination

The findings in such individual cases added to the understanding of existence of a disease in clinical form, even if because of some reasons, serology gives a negative result.

Study of kills means post-mortem examination, in the present context. Detailed post-mortem examination is important for the diagnosis of the diseases. It also gives information about the health of the animal.

Diagnosis of the diseases and parasitism require careful and detailed evaluation of dead animal with subsequent collection, preservation and eventual examination of appropriate specimens.

The field investigator should record all pertinent data that may be of importance in making a diagnosis. Any fact that the investigator feels, might have some bearing, should be recorded. The history should include species, sex, age of the animal, locality from which the animal came, habitat type, date and time when it was located, if the animal is dead, complete description of the clinical signs of illness, number of other animals of the same species involved and died.

Post-mortem examination if at all possible should be conducted in diagnostic laboratory. Where they can be necropsied by trained pathologist. Essential tools required for post-mortem examination are rubber gloves, sharp knives, scissors, bone shears and forceps. It is also desired to have a clean, well lighted placed to work, disinfectant, good camera, adequate water supply, 10% formalin, 70% alcohol, sterile plastic bags, vials for collection of blood and glass slides are necessary material for collection of the specimens.

The post-mortem examination should be done thoroughly and examined all external characters of the carcass. Open the carcass in such a way that the systematic and system wise post-mortem examination can be made with ease.

Summary

In past few decades, there have been outbreaks of many infectious viral and bacterial diseases amongst free-ranging wildlife. While some of these have been reported and published, most events often go unreported and unnotioced. Moreover, it is usually not possible to mount satisfactory disease investigations when mortality has already become evident. The disease does not flow in one direction; wild and domestic animals share many infections that may travel in any direction. Thus it is imperative to understand this dynamics based on the scientific facts. These facts warrant the continuous surveillance and monitoring of various diseases, especially those prevalent or expected in the domestic livestock at wild-domestic interface.

Considering these voids and gaps in the understanding of the disease pattern among wildlfe, this study was undertaken to systematically study the incidence of certain infectious diseases among ungulates of Sariska Tiger Reserve. Sambar was selected as the representative for ungulates in this study

Appendix 1

List of various sera samples collected at STR

Sample No.	Source(Animal species)	Sex and age	Plazce	Remarks
S 1	Sambar (free ranging)	M, A	Kalighati	Apparantly healthy
S2	"	M, A	Kalighati	"
S3	"	F, A	Kalighati	"
S4	"	M, A	Kalighati	"
S5	"	F, A	Kalighati	"
S6	"	F, A	Kalighati	"
S7	"	M, A	Kalighati	"
S8	"	M, A	Kalighati	"
S9	"	F, A	Kalighati	"
S10	"	M, A	Kalighati	"
S11	"	M, SA	Kalighati	"
S12	"	F, A	Kalighati	"
S13	"	M, A	Kalighati	"
S14	"	F, A	Kalighati	"
S15	"	F, A	Khajuron wala nala	"
S16	"	M, A	Kalighati	"
S17	"	F, A	Kalighati	"
S18	"	M, A	Kalighati	"
S19	"	M, A	Kundli road	"
S20	"	M, A	Brihmnath	"
S21	"	M, A	Brihmnath	"
S22	"	M, A	Brihmnath	"
S23	"	M, A	Brihmanth	"
S24	"	M, A	Brihmanth	"
S25	"	F, A	Brihmanth	"
S26	"	M, A	Tarunda	"
S27	"	M, A	Feta ki pal	"
S28	"	F, A	Feta ki pal	"
S29	"	M, A	Feta ki pal	"
S30	"	M, A	Feta ki pal	"
S31	"	M, A	Kalighati	"
S32	"	M, A	Brihmanth	"