

**ASSESSMENT OF LIVESTOCK DISEASES AS AN INDICATOR OF  
RISK TO GREATER ONE HORNED RHINOCEROS (*Rhinoceros  
unicornis*) IN MANAS NATIONAL PARK, ASSAM**

DISSERTATION SUBMITTED TO  
SAURASTRA UNIVERSITY, RAJKOT,  
IN PARTIAL FULFILMENT OF THE MASTER'S DEGREE IN WILDLIFE SCIENCE

BY  
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UNDER THE SUPERVISION OF  
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## CERTIFICATE

This is to certify that **Dr. Debabrata Phukon**, student of Wildlife Institute of India has carried out an original piece of research work entitled “**Assessment of livestock diseases as an indicator of risk to Greater One-horned Rhinoceros (*Rhinoceros unicornis*) in Manas National Park, Assam**” for the partial fulfilment of the M.Sc. Degree in Wildlife Science from the Saurashtra University, Rajkot, India. These investigations were carried out under our supervision from November 2012 to June 2013. We also certify that this research has not been submitted for any other degree to any University.

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## SUMMARY

Disease ecology is a fast emerging discipline in wildlife conservation. There is particular interest for mega herbivore-livestock interface issues. Diseases also play a role in structuring the demography and viability of the wild population, and this could be addressed by quantitative knowledge on the emergence, spread, persistence and evolution of infectious diseases. Re-introduced wild populations mimic the small population which are vulnerable to stochastic events and disease impacts.

The present study focuses on re-introduced population of Greater One-horned Rhinoceros (*Rhinoceros unicornis*) and their sharing of habitat with livestock in Bansbari Range of Manas National Park (MNP), Assam. It was hypothesised that the interaction of rhinos with livestock has potential to contract diseases from the livestock population, and therefore the diversity and magnitude of disease prevalence in livestock is likely to pose serious threat to the rhinos. The study quantified disease distribution pattern and commonality between livestock and rhino population and map disease gradient in MNP during the period (Jan-April, 2013). The study design involved, (a) sampling of livestock for disease prevalence in the fringe villages, (b) mapping of zone of influence (ZOI) of livestock in the park by tracking livestock movement and sampling on systematic plots, and (c) analysis of livestock husbandry practices in the context of disease prevalence. A total of 110 dung and serum samples collected from 11 villages were subjected to parasitological examination, disease diagnosis and screening for blood parasites. 10 transects were walked from the periphery to interior of the park and 46 plots were laid to quantify dung density distribution as a surrogate for disease risk. Rhino dung sample was collected from 9 fresh dung sites inside the park for screening of parasitic load and to understand the commonality between livestock and rhinos. The threat to rhino population was measured as an index, given by disease prevalence  $\times$  livestock use intensity.

Of the 110 biological samples collected from 110 livestock in 11 villages, 91% of animals showed antibodies against one or the other infectious agents. Serodiagnosis report confirmed the presence of antibodies against infectious diseases viz. Blue tongue, Tuberculosis, Brucellosis and Chlamydiosis, however were negative for

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Leptospirosis in the livestock sampled. Blood smears revealed the presence of haemoprotozoan *Babesia bigemina*, and were negative for *Anaplasma*, *Theileria*, *Trypanosoma* and *Pasteurella sp.* (bi-polar organism). 48% of the 110 livestock sampled across 11 villages showed the prevalence of parasites in their dung. The parasites reported from dung samples of livestock include *Haemonchus sp.*, *Paramphistomum sp.*, *Eimeria sp.*, *Toxocara vitulorum*, *Fasciola gigantica*, *Schistosoma sp.*, and *Oesophagostomum sp.*

ZOI as measured from the fringe villages to forest interior ranged from 500m to 3.5kms (avg. dist. 2kms), and accounted for 19sq.kms. area. Interpolation results of dung density indicated significant variation of livestock use within ZOI, with high usage contributed by Gyatigaon followed by Katajhar and Rajabeel. Based on the dung analysis of rhinos, parasitic ova of *Paramphistomum sp.* and significant load of protozoan *Balantidium coli* was observed.

The study established wide prevalence of disease in the livestock. Given that the ZOI of livestock in MNP includes home range of rhinos, the rhino population is likely to be under disease risk and conservation efforts needs to incorporate disease perspective for recovery efforts and long-term viability of rhinos in MNP.

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# 1. INTRODUCTION

## 1.1 Disease Ecology in Wildlife Management

Conservation of wildlife requires an understanding of the population dynamics, community structure, biogeographical patterns and individual behaviour of species. Additionally, health concerns of these populations are now being considered to influence the survival of wild species (Meffe 1999; Daszak et. al 2000). Traditionally, wildlife diseases have been of interest only after they have directly impacted livestock or human health. Disease is defined as “any impairment that interferes with or modifies the performance of normal functions, including responses to the environmental factors such as nutrition, toxicants, and climate; infectious agents; inherent or congenital defects, or combinations of these factors” (Wobeser 1981).

In natural populations of wild animals, infection and disease have been considered as a significant factor affecting the demography and viability of the population. Disease ecologists strive to understand the causes and consequences of parasitic infection, including the emergence, spread, persistence and evolution of infectious diseases. Parasites are ubiquitous in the lives of wild animals and represent a major component of biological diversity (Price 1980).

Evaluating the causation of a disease requires an understanding of multiple features of each of the agents, the host and its environment, a combination of which has been called as ‘the epidemiological triad’. However, this triad is insufficient to describe many of the diseases wherein a web of causation has to be considered having a list of predisposing factors like nutrition, stress, weather, etc. (Wobeser 1994). ‘The concept of iceberg’ fits well to the prevalence of a disease in a wild population, both clinical cases and subclinical cases, comparing it to the tip of the iceberg and the part unobserved beneath the ocean surface respectively. Infectious agents can exert important effects on host population dynamics when they are endemic or epidemic. When endemic, their effects on host reproduction and survival can lead to decrease in host population, whereas epidemics can have a much higher impact than density-dependent effects on wild populations.

Infectious diseases can also cause significant population declines in wildlife, as illustrated by canine distemper virus in Serengeti lions (Roelke-Parker et al. 1996), Ebola outbreaks in African apes (Leroy et al. 2004), and multiple pathogens that affect amphibian populations (Daszak et al. 1999; Pounds et al. 2006). Threatened host populations are predicted to harbour relatively few parasite species as a consequence of limited geographic ranges; small and isolated populations (Lyles & Dobson 1993; Lafferty & Gerber 2002).

Livestock populations in and around protected areas pose a threat to the wildlife of that area by sharing and spreading of diseases. Domesticated animals are maintained at high population densities and have a near-global distribution; they can serve as reservoirs of infectious disease for wild mammals (Lafferty & Gerber 2002).

### **1.1 Livestock and Wildlife Interaction**

The proximity of human habitation in protected areas often results in conflicts between human and wildlife (Rodgers 1989). Livestock in fringe villages of protected areas pose a competition to wildlife with regards to resource sharing by grazing on critical wildlife habitats. It is a well-established fact that overgrazing alters the ecosystem function, structure and organisation by reducing species richness and diversity, increasing the proportion of unpalatable weed species, accelerating soil erosion and depleting the nutrient pool (Namdeo et al. 1989; Putman et al. 1989; Skarpe 1991).

In India land use pattern in the fringe areas and within protected areas involve agriculture, livestock grazing and collection of minor forest products. Livestock grazing is widespread in such areas and form an integral part of the agro-pastoral system. There are various factors that explain the constantly increasing interactions between wildlife and livestock. The growth in human population has resulted in greater use of land, thereby bringing their livestock close to wild populations. In 2004, the World Health Organization, the Food and Agriculture Organization of the United Nations, and the World Organization for Animal Health had a joint conference to discuss upon the increase in disease risk and emergence of diseases not known earlier (WHO/FAO/OIE 2004). A common theme of primary risk factors for the emergence

and spread of emerging diseases was the increasing demand for animal protein, associated with the expansion and intensification of animal agriculture, long-distance live animal transport, live animal markets, bushmeat consumption, and habitat destruction (Greger 2007).

## 1.2 Diseases and Transmission

Diseases at wildlife-livestock interface has caused a growing concern among the scientific community over the past few years due to its impact on three aspects-wildlife, livestock and human health (Karesh et al. 2007). These are the diseases that have been modified due to gradual change in many related factors like environmental and ecological (Martin et al. 2011). Some of the diseases in livestock have “spilled-over” to wild populations and have “spilled back” to livestock. Wildlife can also serve as vectors for non-zoonotic diseases of livestock, potentially devastating the economy and resulting in the loss of human dietary protein (Rhyan et al. 2010).

Many diseases have been reported from around the world that is shared between wildlife and livestock (Table 1.1). In most of the cases, the economic, geographical and ecological situations which permit reciprocal transmission are extremely variable, so the extent of disease surveillance and diagnostic activities also varies (Pastoret et al. 1988). Movement of livestock provide a route for the transmission of pathogens between populations. Regular livestock movements can rapidly spread disease, making early detection crucial for disease control (USGS 2001). Such threat is observed in many of the protected areas in the world where grazing by livestock has created the interface for the spread of disease to wild populations.

Some examples of diseases that have been transmitted from livestock to wildlife are classical swine fever (hog cholera) in wild boars (Artois et. al 2002), myxomatosis and rabbit haemorrhagic disease in wild rabbits (Garcia-Bocanegra et. al 2010), bovine viral diarrhoea (BVD) in roe deer (Casaubon et. al 2012), *Mycobacterium bovis* in wild boars, badgers, and deer (Lisle et. al 2001) ; and brucellosis in a broad range of wildlife in all European countries (Al Dahouk et. al 2007). Serological surveys performed on different free-ranging ungulate species revealed the presence of alpha-Herpes viruses related to Bovine Herpes virus-1 in 7 European countries

(Frolich 2011). Malignant catarrhal fever found in deer in Germany indicated that sheep are the main reservoir species for the virus (Frolich 2002). According to a report by International Livestock Research Institute (ILRI) in February, 2011, the increase in density of livestock production units and poor bio-security in Asia has resulted in epidemics both in human landscape as well as in the wilderness. In most continents, cattle are the most important maintenance hosts for Foot-and-Mouth disease virus (FMDV) (OIE 2012). Wildlife hosts do not seem to be able to maintain FMDV, and are usually infected by contact with livestock. Transmission of bovine tuberculosis (BTB) from livestock to badgers has been reported from United Kingdom, where the intensity of transmission was found to be more in case of badger-cattle than between different badger groups (Bohm et al. 2009). Arthropod vectors also play a major role in the transmission of disease to livestock. The most important diseases transmitted by ticks, particularly in domestic ruminants, are babesioses, theilerioses, anaplasmoses and cowdriosis that can have a great impact on wild populations by depressing their production and transmitting infectious diseases (Jongejan 2004). In Scotland, sheep and feral goats became infected with paratuberculosis (*Mycobacterium paratuberculosis*) after sharing grazing pasture with infected cattle (Ris et al., 1987, 1988). These infected cattle and sheep can shed in excess of  $10^8$  bacilli/ gram of faeces (Cranwell, 1997). The wild herbivores such as rabbits or hares sharing pasture with infected cattle can contract the pathogenic bacteria through the ingestion of faecal contaminated vegetation (Daniels et al. 2003).

A study conducted by Roelke-Parker et. al in 1996 in Serengeti National Park reported an epidemic caused by a morbillivirus closely related to canine distemper virus (CDV) that emerged abruptly in the lion population of the Serengeti National Park, Tanzania, during early 1994. Fatal neurological disease characterized by grand mal seizures, myoclonus encephalitis and pneumonia were reported from affected lions. By August 1994, 85% of the Serengeti lion population had anti-CDV antibodies. The epidemic spread northwards to lions in the Maasai Mara National reserve, Kenya, and even affected hyenas, bat-eared foxes, and leopards. Close phylogenetic relationship between CDV isolates from lions and domestic dogs around the park were noticed (Roelke-Parker 1996). Bovine Tuberculosis (BTB) is another important disease that results from increased interaction between wildlife and

livestock. Bovine tuberculosis was apparently first introduced into African buffalo (*Syncerus caffer*) in the southern region of Kruger National Park (KNP, Republic of South Africa) in the 1960's or 1980's from domestic cattle (Bengis et al., 1996). By the late 1980's, BTB was largely eradicated from the domestic animal populations surrounding KNP (Kloeck, 1998), but inside KNP it apparently persisted undetected until 1990, when it was diagnosed opportunistically in a single African buffalo (Bengis, 1999).

**Table 1. Review of few diseases reported from wildlife/livestock interface:**

Study	Disease	Wild Animals	Livestock	Salient findings
Assessing disease risk at the wildlife–livestock interface: A study of Sierra Nevada bighorn sheep (Clifford et. al 2009)	Respiratory disease	Big horn Sheep	Domestic sheep	The reduction in disease risk to Big horn sheep due to the restricted grazing time of domestic sheep.
Detection of Anaplasma antibodies in wildlife and domestic species in wildlife-livestock interface areas of Kenya by major surface protein 5 competitive inhibition enzyme-linked immunosorbent assay (Ngeranwa et. al 2008)	Anaplasmosis	Eland, blue wildebeest, hartebeest, impala, Thomson's gazelle, Grant's gazelle, giraffe and plains zebra	Cattle, sheep and goat	High levels of antibodies titre in animals from wildlife livestock interface areas.
Assessing risks of disease transmission between wildlife and livestock: The Saiga antelope as a case study (Morgan et. al 2006)	Foot and Mouth Disease, Gastro-intestinal Nematodes	Saiga antelope	Domestic sheep	Mathematical models of wildlife disease can be usefully applied to assess the risk of transmission across the wildlife–livestock interface.
Effect of season on helminth loads of wild herbivores and cattle in the Mudumalai Wildlife Sanctuary, Southern India (Dharmarajan et. al 2005)	Gastro-intestinal helminthiasis	Chital, Indian Bison and Asian Elephant	Cattle	Hosts have shown an increase in helminths load during the rainy season.

An important factor responsible for the outbreak is the direct or indirect (vector) contact of infected animals or populations (wild or domestic) with susceptible populations at the interface of their ranges.

The increase in land-use pressure and grazing of livestock has led to a conflict between pastoralists and wildlife resulting in a growing risk of disease transmission between livestock and wildlife and increasing competition for grazing and water resources (Kock et al. 2002). Most pastoralists survive at subsistence level and have limited access to veterinary services. Disease control measures usually rely on ethno-veterinary practices, based on traditional knowledge of livestock diseases (Wambwa 2012). Livestock herbivores are at constant risk of inter- and intra-specific disease transmission via the faecal-oral route during grazing. Due to the sharing of habitat between livestock and wild populations, the host range of previously confined parasites expands. The expansion in grazing land of livestock has resulted in the sharing of habitats with the wild animals. In tropical countries, due to the high ambient temperature, animals tend to concentrate under trees or other shaded areas for parts of the day like wallowing sites, preferring to graze early in the morning and late in the afternoon (Ayele et al. 2004). These areas serve as interface for transmission of disease to both wildlife and livestock through naso-pharynx or faecal secretion.

Arthropod vectors like ticks and flies play an important role in the bi-directional transmission of infectious agents in wildlife-livestock interface. Ticks transmit a great variety of pathogenic microorganisms, protozoa, rickettsiae, spirochaetes and viruses, than any other arthropod vector group, and are among the most important vectors of diseases affecting livestock and wildlife (Jongejan et al. 2004). The prevalence and distribution of well-known vector-borne diseases have already increased during the last decade due to a result of this interface (Rocque et al. 2008). In the island of New Caledonia the tick *Boophilus microplus* has been introduced without the diseases it transmits elsewhere, namely babesiosis and anaplasmosis, but the change in climatic conditions and a large density of beef cattle and the presence of livestock-wildlife interface has made the cattle population susceptible for disease contraction (Brun et al. 1983). As a matter of fact relatively few species of ticks have successfully adapted to livestock or feed on a human subject, and these have developed into efficient

vectors of a range of pathogenic microorganisms, while virtually all human tick-borne diseases are zoonoses (Jongejan and Uilenberg 2004).

#### **1.4 Rationale for the study**

The presence of disease in a population suppresses the overall health of the population resulting in weak and unhealthy individuals and compromise long term conservation of species. These populations may also serve as maintenance host for the various infectious agents (Doligalska and Donskow-Lysoniewska 2012) and pose threat to other individuals and con-specific species. Parasitic infestations remain a significant problem for individuals in resource-poor settings and require scientific basis for effective management. New concepts regarding the interactions of parasites with host immunity necessitates the need for collection of further epidemiological and clinical data to unravel the complexities of such immunological interactions. Recently built One-horned Rhinoceros population in Manas National park are susceptible to variety of diseases which may result from interaction with livestock which share common habitats. Many of the diseases are common between Rhinos and livestock. Understanding the dynamics of disease transmission at the interface is a critical step in preventing disease transmission and developing effective management strategies to ensure long term survival and conservation of Rhinoceros in the Park. A study addressing above issues is being undertaken with the following objectives.

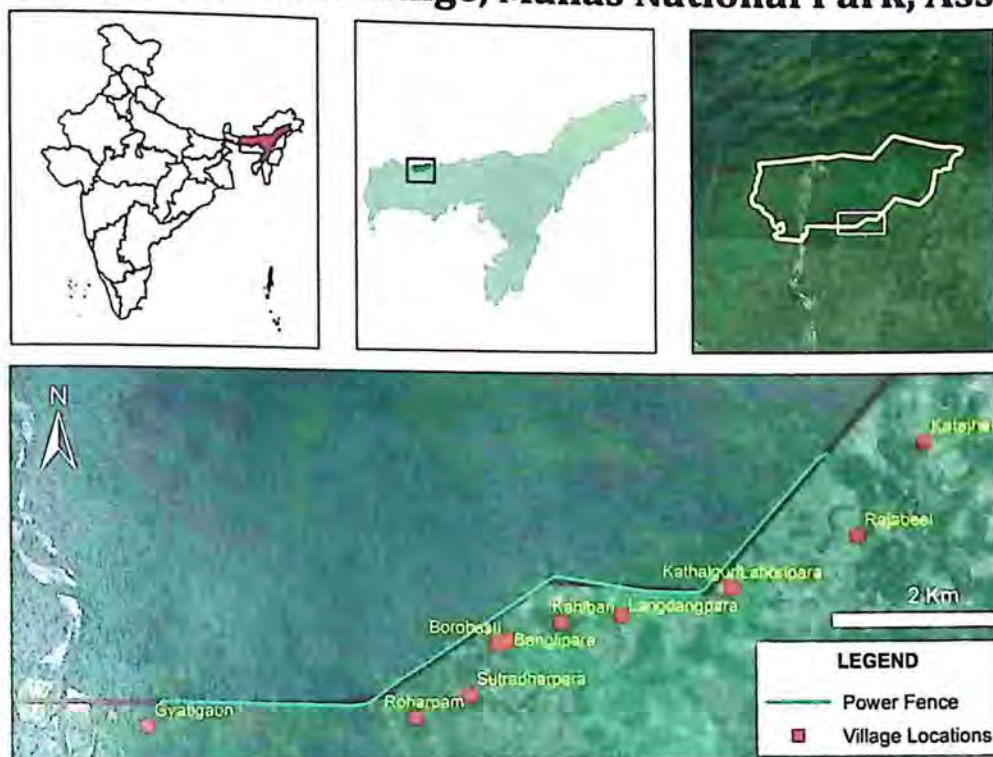
#### **1.5 Objectives**

1. To assess the disease distribution pattern and commonality between livestock and rhino population in Manas National Park, Assam.
2. To map disease risk gradients in Manas National Park.

#### **1.6. Study Area**

Physical Description: Manas National Park (26°45' – 26°26'N, 91°59' - 91°26'E) embracing part of Chirang and Baksa districts of Assam, north-eastern India, provides a suitable habitat to the Indian Rhinos (Map 1). Manas NP lie at the base of the Bhutan-Himalayas with unique biodiversity and landscape.

## Study Area Map showing Fringe Villages and Power Fence Boundary in Bansbari Range, Manas National Park, Assam



**Map 1. Study Area showing fringe villages.**

It is one of the first reserves included as tiger reserve under Project Tiger in 1973 and is a World Heritage Site. It extends over an area of 500 sq. km. from Sankosh River in the west to Dhansiri River in the east. The area on an average is flat and slopes from the north to the south. A number of rivers flow through the park in the north – south direction. The Manas - Beki system are the major rivers that flow through the park dividing it into two distinct parts. This river system along with other rivers running through the park often change courses and result in flash flood during the monsoon season. These rivers also carry an enormous amount of silt and rock debris from the upstream areas aided by the heavy seasonal rainfall, fragile nature of the rock formations and steep channel gradients. This leads to the formation of alluvial terraces, comprising deep layers of deposited rock and detritus overlain with sand and soil of varying depth, shifting river channels and swamps. The area under the Manas – Beki basin, is at times inundated during the monsoon but never for very long due to the sloping relief ([www.manasassam.org/worldheritage.asp](http://www.manasassam.org/worldheritage.asp)).

The park area is divided into three ranges, the western range is Panbari, the eastern range is Bhuyanpara and the most central range is Basbari. The Panbari extends from

river Sukhanjan to river Manas and Bhuyanpara from river Pota to river Rupahi and the remaining central portion is covered by the Basbari range.

The study site for the research is the central range, Bansbari Range, which is the only range having a continuous stretch of 10kms length solar-powered fence with villages in the fringe area. This results in specific entry points of livestock inside the park from each village. The sites of interest in this project are these fringe villages comprising of around 11 villages/hamlets where wide arrays of mostly non-descript livestock are reared. The local livestock rearing system is largely based on free-range livestock rearing methods. This suggests that the rhinos are potentially at risk resulting from pathogen transmission between the two populations.

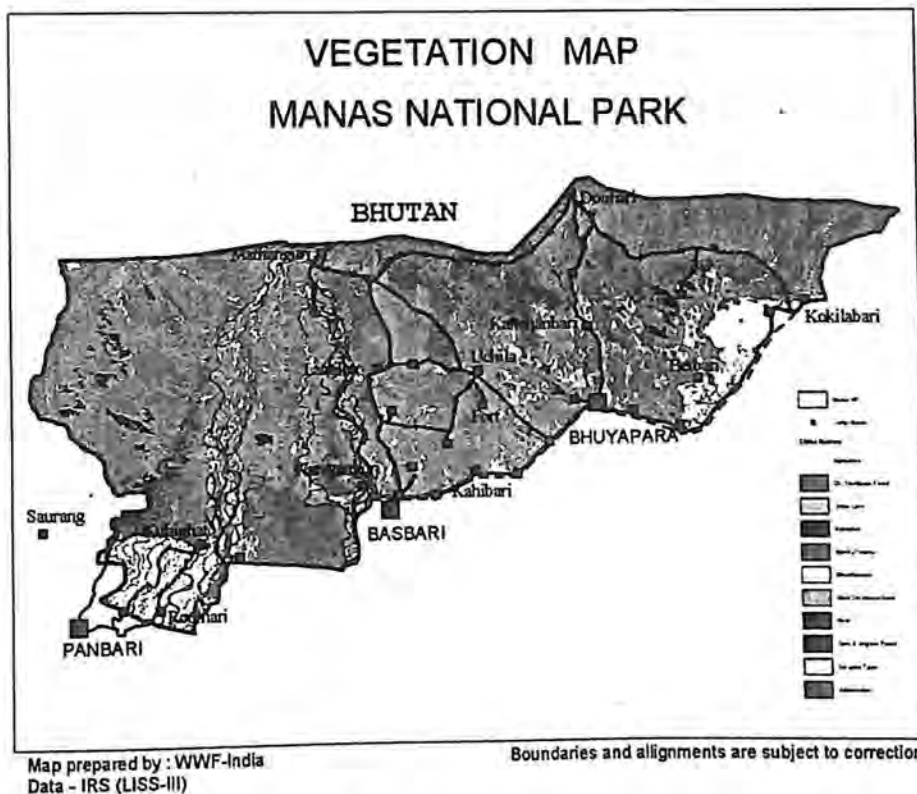
**Climate:** The Park experiences a warm and humid climate with heavy rainfall during the monsoon season normally extending from May to September. The mean maximum summer temperature is 37°C and the mean minimum winter temperature is 11°C. Mean annual rainfall ranges from 3332mm at Batabari to 4489mm at Kachugaon, based on 11 and 17 years of records, respectively ([www.manasassam.org/worldheritage.asp](http://www.manasassam.org/worldheritage.asp)).

**Fauna:** The Park has recorded 55 species of mammals, 380 species of birds, 50 of reptiles, and 3 species of amphibians. Out of these wildlife, 21 mammals are listed in India's Schedule I mammals and 31 of them are threatened. Manas houses more than 450 species of birds and has the largest population of endangered Bengal Florican.

**Flora:** The Park since historical days is very rich in terms of floral composition and records an impressive collection of more than 600 species. Three main types of vegetation are identifiable-

- (a) Tropical semi-evergreen forests primarily to the northern part, common trees includes *Aphanamixis polystachya*, *Anthocephalus chinensis*, *Syzygium cumini*, *S. formosum*, *S. oblatum*, *Bauhinia purpurea*, *Mallotus philippensis*, *Cinnamomum tamala* and *Actinodaphne obvata*;

- (b) Topical moist and dry deciduous forests is the most common type. This forest is characterized by trees such as *Bombax ceiba*, *Sterculia villosa*, *Dillenia indica*, *D. pentagyna*, *Careya arborea*, *Lagerstroemia parviflora*, *L. speciosa*, *Terminalia bellerica*, *T. chebula*, *Trewia polycarpa*, *Gmelina arborea*, *Oroxylum indicum* and *Bridelia* spp.; and
- (c) Grasslands distributed mainly to the southern parts (Map 2), comprising many different grass species together with a variety of tree and shrub species (e.g. *Dillenia pentagyna*, *Phyllanthus emblica*, *Bombax ceiba*, and species of *Clerodendrum*, *Leea*, *Grewia*, *Premna* and *Mussaenda*). There is also a considerable variety of aquatic flora along river banks and in the numerous pools ([www.manasassam.org/worldheritage.asp](http://www.manasassam.org/worldheritage.asp)). Dry deciduous forests represent early stages in succession and are replaced by moist deciduous forests away from water courses, which, in turn, are succeeded by tropical semi-evergreen climax forest. Grasslands cover about 50% of the park. Some 393 species of dicotyledons, including 197 trees, and 98 species of monocotyledons have been identified ([www.manasassam.org/worldheritage.asp](http://www.manasassam.org/worldheritage.asp)).



**Map 2. Vegetation Map of Manas National Park, Assam (Courtesy: WWF-India).**

## 1.7. Study Animal: Greater One-Horned Rhinoceros (*Rhinoceros unicornis*)

### Taxonomy

Kingdom	Animalia
Phylum	Chordata
Class	Mammalia
Order	Perissodactyla
Family	Rhinocerotidae
Scientific Name	<i>Rhinoceros unicornis</i>
Species Authority	Linnaeus 1758

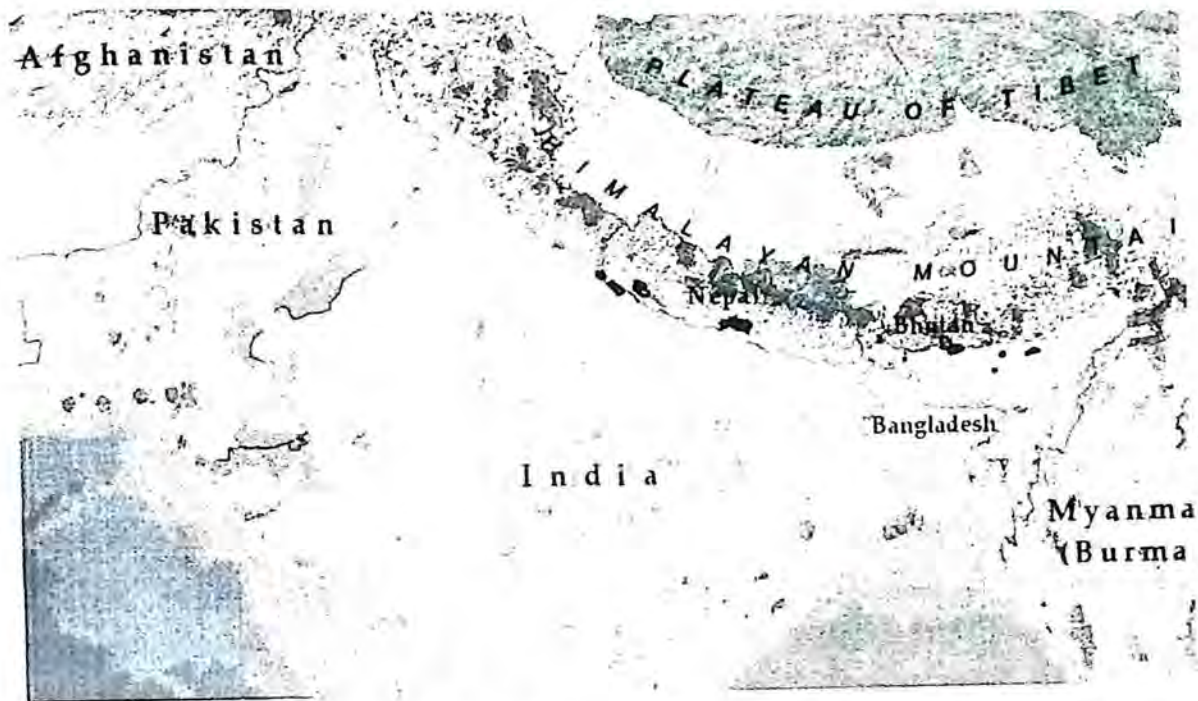
### Biology:

Greater one-horned rhinos have a brownish-grey, hairless skin, which develops thick folds, resembling armour plating. They weigh between 1,800 - 2,700 kg, shoulder height is 1.75 - 2 metres, and are 3- 3.8 metres long. Females are sexually mature at 5-7 years of age; males at 8-10 years. Mating takes place throughout the year and there is no specific calving season. Their gestation period is approximately 16 months (465-490 days), and they give birth every three years. The interval between births ranges from 34-51 months. The birth weight ranges from 60-77 kg (Srivastav and Nigam 2009). Greater one-horned rhinos are grazers, although occasionally they consume browse. When not grazing on land, they like to immerse themselves in water, where they also graze on aquatic grass-like plants. This species is the most amphibious of the living rhinos. The rhino wallows in lakes, rivers and temporary pools. Heat regulation and escape from flies is probably a major function of wallowing. Seasonal variations in resource availability results in regular movements of rhinos between habitat types. Rhinos are predominantly solitary, although temporary aggregations at wallows and feeding grounds are fairly frequent.

### Distribution:

Greater One-horned Rhinoceros (*Rhinoceros unicornis*) is an obligate species of grassland habitat. The historic distribution of the Indian or Greater one-horned rhino *Rhinoceros unicornis* Linné, 1758, covered the huge floodplains of the Indus, Ganges and Brahmaputra on the Indian subcontinent (Pakistan, India, Nepal, Bhutan, Sikkim, Assam, Bangladesh, and Myanmar). The species inhabits the riverine grasslands of the Terai and Brahmaputra Basins (Foose and van Strein 1997). The present day

population is restricted to two small areas in the northeast of their former range, one in southern Nepal and one in Assam (Map 3). The total population estimates in May 2007 were 2,575 individuals, with a total of 378 in Nepal and 2,200 in India (Asian Rhino Specialist Group 2007).



**Map 3. Present distribution of Greater One-horned Rhinoceros (*Rhinoceros unicornis*) showing protected areas (Courtesy: Talukdar, B.K., Emslie, R., Bist, S.S., Choudhury, A., Ellis, S., Bonal, B.S., Malakar, M.C., Talukdar, B.N. & Barua, M. 2008. *Rhinoceros unicornis*. In: IUCN 2012. IUCN Red List of Threatened Species).**

#### *Conservation initiatives:*

The conservation and protection of this majestic species has been of great importance since the beginning of the last century as it was severely threatened. Extensive hunting during 1908 in the Brahmaputra Valley, Assam resulted in as low as 20 individuals in the area of today's Kaziranga National Park. The species is categorised as Vulnerable B1 ab (iii) ver 3.1 in the Red List Category of IUCN published in 2008. It is listed in Schedule 1 of the Wildlife Protection Act (1972), Government of India, and in CITES Appendix 1 since 1975. The restricted range of distribution, loss of habitat, genetic bottleneck that the species has gone through and the still relatively small size of the slowly recovering population makes the Indian rhinos still vulnerable to inbreeding problems and epidemic diseases.

Small population of rhinos naturally thrived in Manas National Park (MNP) as the area provided excellent habitat for the Rhinos. Violent civil conflict during 1989 resulted in massive damage to the park's infrastructure, including destruction of anti-poaching camps, roads and villages along with rampant poaching resulted in species extinction from the area. The last rhino was seen in Manas in 1996. As a population building exercise and providing second home to Rhinos, a reintroduction program was initiated in 2006 in MNP with international and national collaboration. Under Indian Rhino Vision-2020 programme (IRV-2020); eighteen rhinos were translocated from Pobitora Wildlife Sanctuary and Kaziranga National Park, to establish the species in MNP. The IRV-2020 envisages in increasing the total rhino population to 3000 in Assam by the year 2020. MNP is one of the first such protected areas (PA) where rhinos have been translocated from wild to wild under IRV-2020. Though the program has taken care of the major reasons for the extinction, this reintroduced population is vulnerable to diseases that may be contracted from livestock which share similar habitats. In addition, five rhinos are also there in the park under the Rehabilitation Program, all rescued from Kaziranga NP.

#### *Diseases in Rhinos:*

Rhinos share habitats with other wildlife species as well as livestock are likely to be exposed to infectious and/or contagious agents (Fink, 1982). Review of literature on diseases reported in rhinoceros is provided below:

- A study conducted by Chakraborty and Gogoi, 1996 at Assam State Zoo revealed variety of parasites affecting One horned Rhinoceros. The major parasites reported included nematodes (hookworms- *Necator* and *Bunostomum*)
- Mondal and Manna, 2012 reported *Rhinoceronema unicornensis* gen. n., sp. n. (Cosmocercoidea: Atractidae) from Indian one horned Rhinoceros (*Rhinoceros unicornis* Linnaeus, 1758) maintained at the Alipore Zoological Garden, Kolkata,
- Serosurvey carried out by Fischer-Tenhagen *et. Al*, 2000 from South Africa revealed presence of antibodies for 16 different infectious agents in free-ranging black and white rhinoceros in Africa

- A documentation of parasites of African rhinos was carried out by Penzhorn *et. Al*, 1994 and reported presence of *Babesia bicornis* and *Theileria bicornis*.
- Brief review of diseases reported in rhinoceros from Brahmaputra Valley, Assam included following:
  - Anthrax (Kaziranga NP, Lahan and Sonowal, 1973) - sporadic cases.
  - Haemorrhagic Septicaemia (Kaziranga NP, Hindustan Times 19th August, 1980) - epidemic case
  - Some of the endoparasites reported from Kaziranga NP – Paramphistomum, Strongyle, Coccidia, and Anoplocephala (Chakraborty and Islam, 1993), Chabertia sp. (Rhino Resource Centre, 2009).

### 1.8. Research Hypotheses:

#### Objective:

To understand the livestock disease distribution pattern in the fringe villages of the park.

#### Hypothesis 1-

Distribution of diseases in the livestock of the fringe villages of Manas NP show uniform pattern across the villages, suggesting that all livestock present equal risk to rhinos.

#### Alternative hypothesis-

Occurrence of diseases in the livestock is a function of varying husbandry practices. E.g. feeding regimes, veterinary care, and socio-economic attributes.

#### Objective:

To understand the zone of influence of livestock inside the park.

#### Hypothesis 2-

Movement of livestock inside the park and disease risk show random pattern.

### **Alternative hypotheses-**

1. Livestock movement inside the park is limited to certain distance from the periphery (zone of influence).
2. Intensity of livestock use of the area and intensity of disease is either linear/non-linear, and these show selection to certain habitat parameters.

### **Objective:**

To understand the level of rhino-livestock interaction inside the park.

### **Hypothesis 3-**

Parasitic load of rhino population is independent of livestock use of the area.

### **Alternative hypotheses-**

1. Higher parasitic load is likely in the rhinos that have home range overlapping on the high intensity of livestock use.
2. Higher parasitic load is likely in the rhinos that have home range with the livestock showing high disease risk.

## 2. METHODS

### 2.1 Disease prevalence in livestock

A cross-sectional survey (Ortiz-Pelaez et. al 2010) was carried out in the fringe villages of Bansbari Range of Manas National Park, Assam, to estimate the prevalence of diseases in livestock. The study area covered eleven villages/hamlets of the fringe area: Katajhar, Rajabeel, Kathalguri, Langdangpara, Lahoripara, Kahibari, Banglipara, Borobasti, Sutradharpara, Roharpam and Gyatigaon (Map).

At selected sampling sites, 10 cattle (~20%) were sampled from each village, 1 cattle per household (household being the sample unit). It was found that there was not much variation in the number of households in each of the fringe villages, so random number of 20% households were generated from the voter's list of each village in MS Excel. Questionnaires were administered to the livestock owners of the sampled animals in order to collect data on socio-economic aspects, management practice and location of household during the study period (Fig. 1).



**Fig.1** Data collection on socio-economic aspects, management practice and location of household during the study period.

### 2.1.1 Blood collection and examination

A total of 110 serum, blood smears and dung samples were collected from cattle across eleven sampled villages/hamlets in vacutainers (Fig. 2). Serum samples were extracted and preserved in 1:10000 thiomersal (trade name - Merthiolate) and stored at freezing temperature prior to laboratory analysis. Serum samples were screened for presence of Brucellosis, Tuberculosis, Leptospirosis, Chlamydiosis and Blue Tongue antibodies using Dot Enzyme Linked Immunosorbent Assay (ELISA) based rapid diagnostic kits (BIONOTE™) directed against the antigen in accordance with the OIE recommendation for testing large numbers of sera.

The blood smears were prepared by fixing them with methanol (100%) in microslides which were later tested for the presence blood borne bacterial disease (Bi-polar organisms-*Pasteurella*) and haemoprotozoans viz. *Babesia*, *Anaplasma*, *Theileria* and *Trypanosoma sp.* by staining them with Giemsa's stain and observed under microscope.



**Fig.2** Blood collection aseptically from the jugular vein of each animal (~10ml).

**Giemsa Staining procedure (Winzler Lab., University of California San Diego):**

Reagents and equipments: Giemsa stain; [LabChem Inc.], 100% Methanol, tissue paper, microscope with 100X oil immersion lens and 10x10 grid eyepiece, immersion Oil (Cedar oil)

**Protocol:** The blood smears were fixed in 100% methanol for 30 minutes followed by rinsing with tap water. A fresh solution of 10% Giemsa stain was prepared in distilled water and left on the fixed smears for 30 minutes. The slide was rinsed using tap water and dried using tissue by gentle dabbing. The slides were then observed under microscope using 100X oil immersion lens and 10x10 grid eye-piece by putting cedar oil. The smear was subjected to 100 fields of observation under the microscope before giving the result.

**2.1.2 Dung collection and coprological examination**

Fresh dung samples were collected from each cattle and preserved in 10% formaldehyde in plastic vials. These were examined for the presence of parasites or their ova (Dryden et. al 2006). The laboratory analysis was performed employing standard protocols of floatation and sedimentation methods for the presence of cestodes, nematodes, trematode eggs and protozoan ova.

**Floatation Method (FAO & ILRI):**

**Equipment-** Two beakers, tea strainer, measuring cylinder, fork, tongue blades, stirring rod, Test tube, microscope, microslides and cover slips, flotation fluid (Saturated Salt Solution).

**Procedure-** Approximately 3 g of faeces was put into container 1 and 50 ml of flotation fluid was then poured. The contents are mixed thoroughly with a stirring device and the resultant faecal suspension was poured through a tea strainer into container 2. The container was left undisturbed for 10 minutes. A drop of the supernatant is transferred to a microslide and observed under the microscope.

**Sedimentation Method (for trematode eggs) (FAO & ILRI):**

**Equipment-** Beakers or plastic containers, tea strainer or cheesecloth, measuring cylinder, stirring device (fork, tongue blade), test tubes, test tube rack, microslide and cover slips, balance or teaspoon, and microscope.

**Procedure-** Approximately 3 g of faeces is measured into Container 1. 40-50 ml of tap water is poured into Container 1. The suspension is mixed (stir) thoroughly with a stirring device (fork, tongue blade). The faecal suspension is filtered through a tea strainer or double-layer of cheesecloth into Container 2. The filtered material is poured into a test tube. The sediment is allowed to stand for 5 minutes. The supernatant is removed (pipette, decant) very carefully. The sediment is re-suspended in 5 ml of water. The sediment is allowed to stand for 5 minutes. The supernatant is discarded (pipette, decant) very carefully. The sediment is transferred to a microslide and covered with a cover slip and observed under microscope.

### **2.1.3 Analysis**

Based on number of livestock showing disease prevalence and management practices, drinking source of livestock, procurement source of livestock and type of treatment (traditional/ veterinary) provided to the livestock, generalized linear mixed model with a logit link function was fitted. The response variable was the count of positive disease prevalence samples and expressed as a binomial distribution to account for the variation in the number of animals tested. The following covariates were introduced in the multivariable model as predictors:

(i) Management practices, (ii) drinking source of livestock, (iii) procurement source of livestock, and (iv) type of treatment employed to the livestock (Ortiz-Pelaez et. al 2010).

### **2.1.4 Livestock parasite richness**

The significance of different variables on richness of parasites and infectious agents were analysed by student's t-test.

### **2.2. Livestock zone of influence (ZOI) data**

Major entry points of livestock into the park were detected through a reconnaissance survey in the park boundary. This was also observed through informal discussion with the livestock owners and the GPS co-ordinates were recorded of each entry point. The livestock herd were followed from each of these major entry points for the whole day and the track was recorded using a Garmin e-trex HCx G.P.S. device. This operation was conducted to generate a surface of zone of influence (ZOI) by the livestock inside the park. The livestock routes were plotted in ArcGIS software and using the interpolation technique the ZOI was generated taking 500 metres buffer for each route (Map 4).



Map 4. Zone of influence (ZOI) of livestock in Bansbari Range, Manas NP.

### 2.2.1 Livestock occupancy data inside the park

Transects were walked from the periphery to the inside of the park along the 10 kilometres southern boundary at 1 kilometre interval. The length of these transects corresponded to the nearest major livestock route (Map 5). After every 500 metres, a circular plot of 25 metres radius was plotted within which livestock dung count was done to assess the livestock use intensity inside the park. Habitat parameters viz. type of habitat, vegetation cover, presence of water body, presence of weed and presence of wild animals was also recorded. This was followed for each transect and G.P.S. co-ordinates were recorded.



Map 5. Map showing the sampling strategy for livestock dung density.

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The livestock dung count for each transect was pooled and the mean was calculated in MS Excel. These were plotted in a graph to show the pattern of livestock use intensity inside the park against the increase in distance from the periphery of the park. The disease prevalence in the adjoining village (s) and livestock use intensity inside the park was ranked, and the multiplied value of these was used to predict the threat to the rhino population inside the park.

***Disease prevalence × Livestock use intensity = Index of threat to rhino population.***

This Index value was regressed with free-ranging of livestock in adjoining village and presence of water bodies inside the ZOI.

### **2.3 Rhino-livestock interaction**

Fresh dung samples were collected from different rhino defecation sites and preserved in 10% formaldehyde in plastic vials. These samples were examined for quantifying the parasitic load of gastro-intestinal parasites using McMaster egg counting technique (epg or egg/gram of faeces) (Gibbons et. al 2010).

#### **McMaster Egg Counting Technique:**

**Equipments-** Two beakers or plastic containers, balance, tea strainer, cheese cloth or dental napkin, measuring cylinder, stirring device (fork, spatula, tongue depressor), Pasteur pipettes and rubber teats, flotation fluid (choice of solution dependant on species expected to be present and availability of reagents), McMaster counting chamber, compound microscope.

**Procedure-** 4 grams of faeces was weighed and placed into a container and mixed with 56ml of saturated salt solution. The content was stirred thoroughly with a spatula. The faecal suspension was filtered through a tea strainer into the second container. The filtrate was stirred with a Pasteur pipette. A sub-sample was withdrawn using the pipette as the filtrate was being stirred. Fluid was stirred and first compartment of the McMaster counting chamber was filled with the sub sample. Fluid was stirred again and second chamber was filled with another sub sample. The counting chamber was allowed to stand for 5 minutes to allow the eggs to float to the surface. The subsample of the filtrate was examined under the compound microscope at 10 x 10 magnification. All eggs within the engraved area of both chambers were identified and counted.

The number of eggs per gram (epg) is calculated as follows:

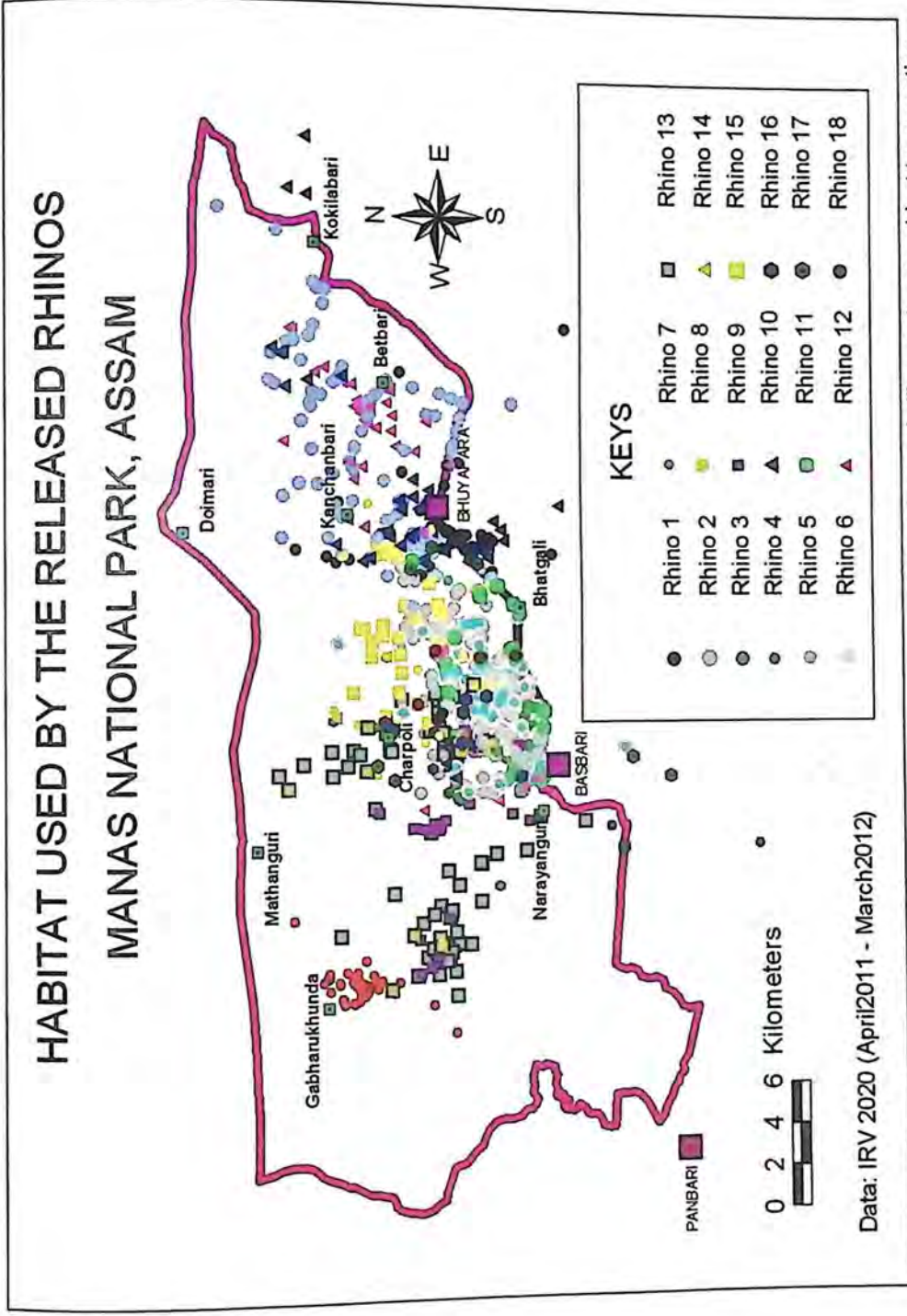
The number of eggs within the grid of each chamber was counted, and those outside the squares were ignored. The total was multiplied by 50, which gives the eggs per gram of faeces (epg).

### 2.3.1 Rhino Home range

The home range of the re-introduced rhinos generated from regular movement monitoring data and 95% Minimum Convex Polygon (MCP) and Kernel Estimates were generated in Geographical Information System (GIS) (AREAS NBL Program, WWF-India) (Map 7). This data was used to determine the home ranges lying inside the ZOI. Greater One-horned rhinoceros have home range of males being usually 2 to 8 km<sup>2</sup> (0.77 to 3.1 sq mi) and overlapping each other (Talukdar et al. 2008). Therefore, the average home range was taken and 1.3 km radius (area =  $\pi r^2$ ) buffer is plotted around each rhino dung site and average dung density against each plot was calculated (Map 5). This would help in assessing the relation between livestock dung density and rhino parasitic load in each sampled rhino dung site. A total of 9 fresh rhino dung samples were collected from different sites for parasitic load.



Map 6. Map showing 1.3 km buffer around rhino dung sites.



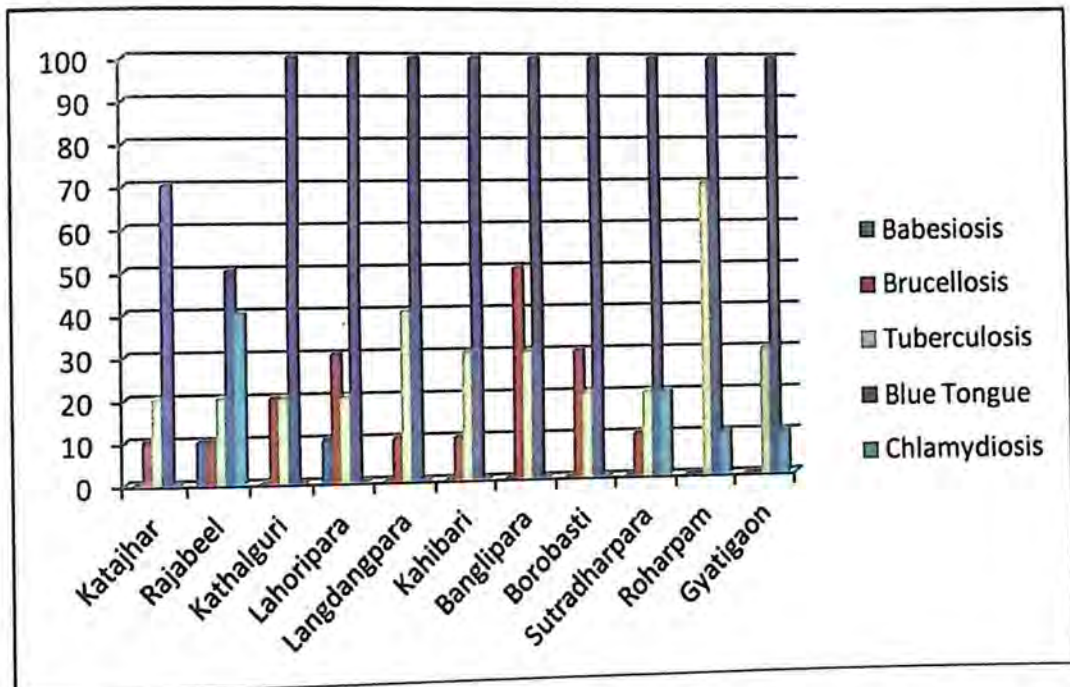
**Map 7. Habitat use by the re-introduced rhinos in Manas National Park (Courtesy: AREAS NBL Program, WWF-India).**

**2.3.2 Reference:** The reference sections for papers cited in this study have been presented in Journal of Biosciences, Bangalore, format and proper guidelines have been followed.

### 3. RESULTS

Laboratory examination of the serum samples collected from livestock reported the presence of antibodies against infectious diseases viz. Blue tongue (91%), Tuberculosis (32%), Brucellosis (18%) and Chlamydiosis (8%) among the livestock population (Fig.3).

#### 3.1 Livestock disease prevalence data



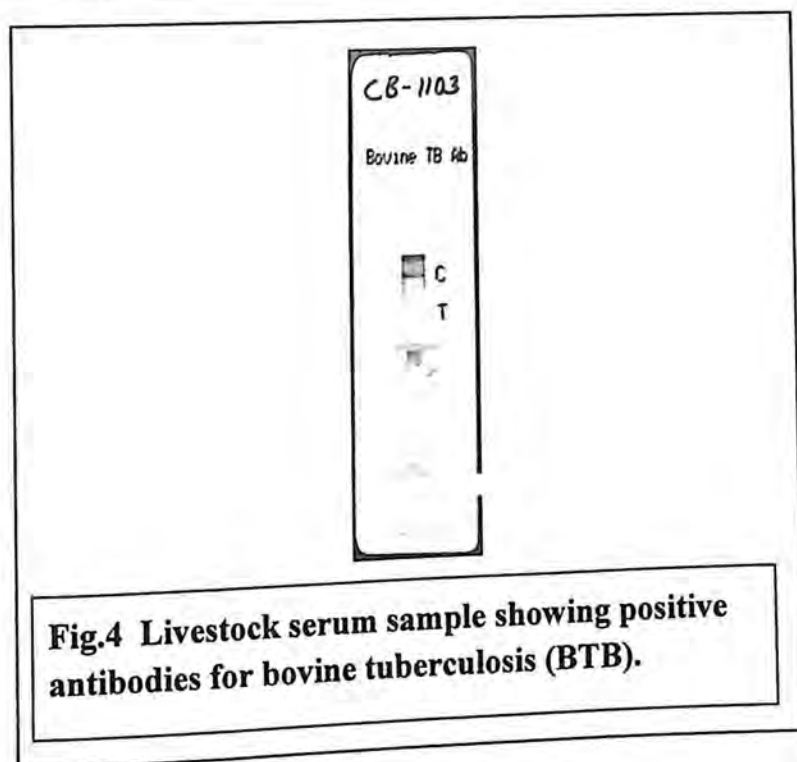
**Fig. 3** Percentage of infectious diseases in fringe villages of Bansbari Range, Manas NP.

**Table 2. Prevalence of nematodes in the fringe villages of Bansbari Range, Manas National Park (N=10 for each village).**

Village	<i>Haemonchus</i> <i>sp.</i>	<i>Paramphistomum</i> <i>sp.</i>	<i>Eimeria</i> <i>sp.</i>	<i>Toxocara</i> <i>vitulorum</i>	<i>Fasciola</i> <i>gigantica</i>	<i>Schistosoma</i> <i>sp.</i>	<i>Oesophagostomum</i> <i>sp.</i>
Katajhar	0	2	1	1	0	0	0
Rajabeel	0	6	0	2	0	0	0
Kathalguri	2	3	2	0	0	0	0
Langdangpara	1	2	0	0	0	1	0
Lahoripara	0	4	0	0	1	0	1
Kahibari	0	2	0	1	1	0	0
Banglipara	0	3	0	0	0	0	0
Borobasti	1	2	1	0	0	0	0
Sutrardharpara	2	1	0	0	0	0	0
Roharpam	1	3	0	0	0	0	0
Gyatigaon	0	3	0	0	1	1	0

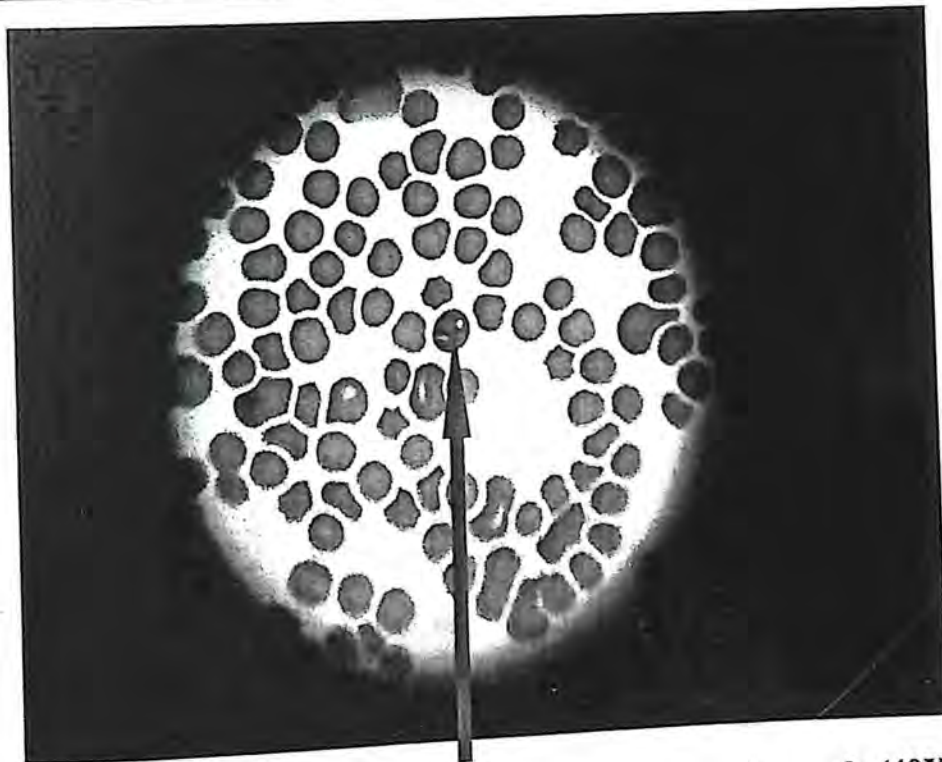
**Table 3. Prevalence of infectious diseases in the fringe villages of Bansbari Range, Manas National Park (N=10 for each village).**

Village	Leptospirosis	Brucellosis	Tuberculosis	Blue tongue	Chlamydiosis
Katajhar	0	1	2	6	0
Rajabeel	0	1	2	5	4
Kathalguri	0	2	2	10	0
Langdangpara	0	3	2	10	0
Lahoripara	0	1	4	10	0
Kahibari	0	1	3	10	0
Banglipara	0	5	3	10	0
Borobasti	0	3	2	10	0
Sutradharpara	0	1	2	10	2
Roharpam	0	0	7	10	1
Gyatigaon	0	0	3	10	1



**Table 4. Prevalence of haemoprotozoan in the fringe villages of Bansbari Range, Manas National Park (N=10 for each village).**

Village	<i>Babesia bigemina</i>	<i>Theileria sp.</i>	<i>Anaplasma sp.</i>	<i>Trypanosoma sp.</i>
Katajhar	0	0	0	0
Rajabeel	1	0	0	0
Kathalguri	0	0	0	0
Langdangpara	1	0	0	0
Lahoripara	0	0	0	0
Kahibari	0	0	0	0
Banglipara	0	0	0	0
Borobasti	0	0	0	0
Sutradharpara	0	0	0	0
Roharpam	0	0	0	0
Gyatigaon	0	0	0	0



**Fig.5 *Babesia bigemina* in RBC of livestock blood sample (40X).**

The prevalence of infectious diseases was regressed with the husbandry practices present in the different households in the sampled villages using a binary logistic function. The disease prevalence in the livestock is the response variable and the different husbandry components are the predictor variables (Ortiz-Pelaez et. al 2010).

Binary logistic: The significance of predictor variables on the response variable were tested using Generalised Linear Model (GLM). According to the AICC values from the result of binary logistic regression, none of the univariate models as well as global model (Table 5) was selected because their AICC values were higher than the null model. Two of the models (M6\_Mrkt+Same\_vill) and (M7\_River+Vill\_pond) having AICC value less than the null model were also not selected because their influence on the response variable was not significant (Table 6 & 7).

**Table 5. Candidate models with AICC values for disease prevalence.**

Model	AICC	$\Delta$ AICC
M1_Free	50.675	-2.067
M2_River	48.739	-0.131
M3_Mrkt	50.402	-1.794
M4_Vety	50.57	-1.962
M5_Free+Both	52.304	-3.696
M6_Mrkt+Same_vill	47.572	1.036
M7_River+Vill_pond	45.934	2.674
M8_Vety+Trad	52.578	-3.97
M9_Global	53.868	-5.26
M10_Null	48.608	0

**Table 6. Parameter Estimates for disease prevalence against husbandry practice.**

Parameter	B	Std. Error	95% Wald Confidence Interval		Hypothesis Test		
			Lower	Upper	Wald Chi-Square	df	Sig.
(Intercept)	17.051	1.4272E4	-27955.147	27989.249	.000	1	.999
[Livst_Mrkt=0]	-19.270	1.4272E4	-27991.468	27952.927	.000	1	.999
[Livst_Mrkt=1]	0 <sup>a</sup>	.	.	.	.	.	.
[Livst_Same_vill=0]	-20.347	1.4272E4	-27992.544	27951.851	.000	1	.999
[Livst_Same_vill=1]	0 <sup>a</sup>	.	.	.	.	.	.
(Scale)	1 <sup>b</sup>						

Dependent Variable: Disease\_pr

Model: (Intercept), Livst\_Mrkt, Livst\_Same\_vill

a. Set to zero because this parameter is redundant.

b. Fixed at the displayed value.

**Table 7. Parameter Estimates for disease prevalence against drinking source of water.**

Parameter	B	Std. Error	95% Wald Confidence Interval		Hypothesis Test		
			Lower	Upper	Wald Chi-Square	df	Sig.
(Intercept)	-3.258	.5883	-4.411	-2.105	30.666	1	.000
[River=0]	-20.007	1.8682E4	-36636.947	36596.932	.000	1	.999
[River=1]	0 <sup>a</sup>	.	.	.	.	.	.
[Source_vill=0]	2.054	.8829	.324	3.785	5.413	1	.020
[Source_vill=1]	0 <sup>a</sup>	.	.	.	.	.	.
(Scale)	1 <sup>b</sup>						

Dependent Variable: Disease\_pr

Model: (Intercept), River, Source\_vill

a. Set to zero because this parameter is redundant.

b. Fixed at the displayed value.

**Correlation:** The predictor and dependent variable was plotted in MS Excel to determine correlation co-efficient. Only Brucellosis has a strong positive correlation with livestock procured from market (r 0.614, p 0.045) and a negative correlation with livestock procured from same village (r -0.865, p 0.001) (Table 8).

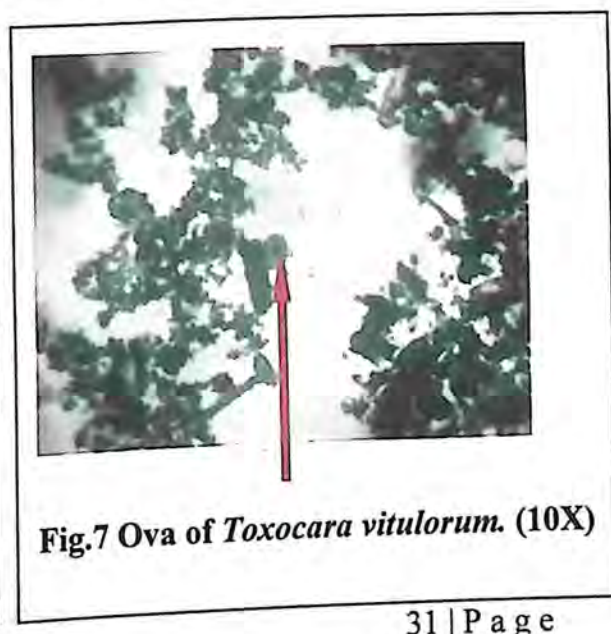
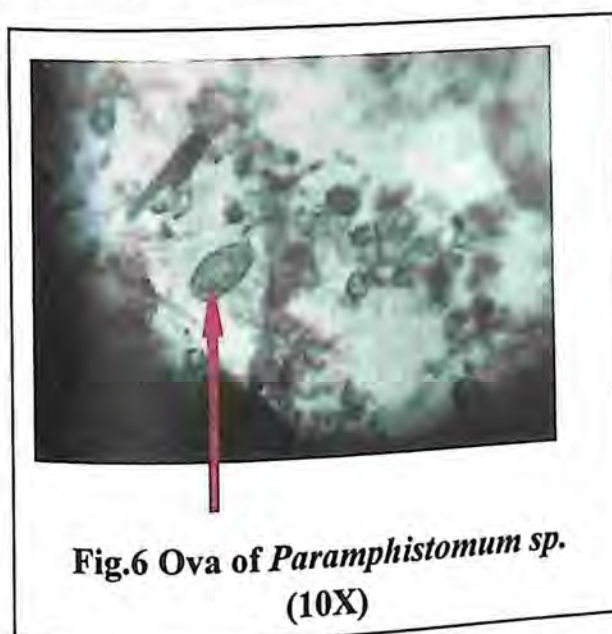
**Table 8. Table showing correlation of brucellosis prevalence to free-ranging livestock and those procured from market.**

VARIABLE	STAT	Free	Both	Livst_Mrkt	Livst_Same vill	Traditnl	Vety
Nematode richness	Correlation Coefficient	-.270	.314	-.172	.476	.485	.359
	Sig. (2-tailed)	.423	.348	.612	.139	.131	.278
<i>Babesia bigemina</i>	Correlation Coefficient	-.492	.376	.189	0.000	-.226	.530
	Sig. (2-tailed)	.124	.254	.577	1.000	.505	.093
Brucellosis	Correlation Coefficient	-.643*	.544	.614*	-.865**	-.384	.149
	Sig. (2-tailed)	.033	.084	.045	.001	.243	.663
Tuberculosis	Correlation Coefficient	.400	-.200	-.387	.570	.207	-.015
	Sig. (2-tailed)	.223	.556	.240	.067	.541	.964
Diseases	Correlation Coefficient	-.238	.294	.273	-.234	.009	.280
	Sig. (2-tailed)	.481	.380	.417	.488	.978	.405

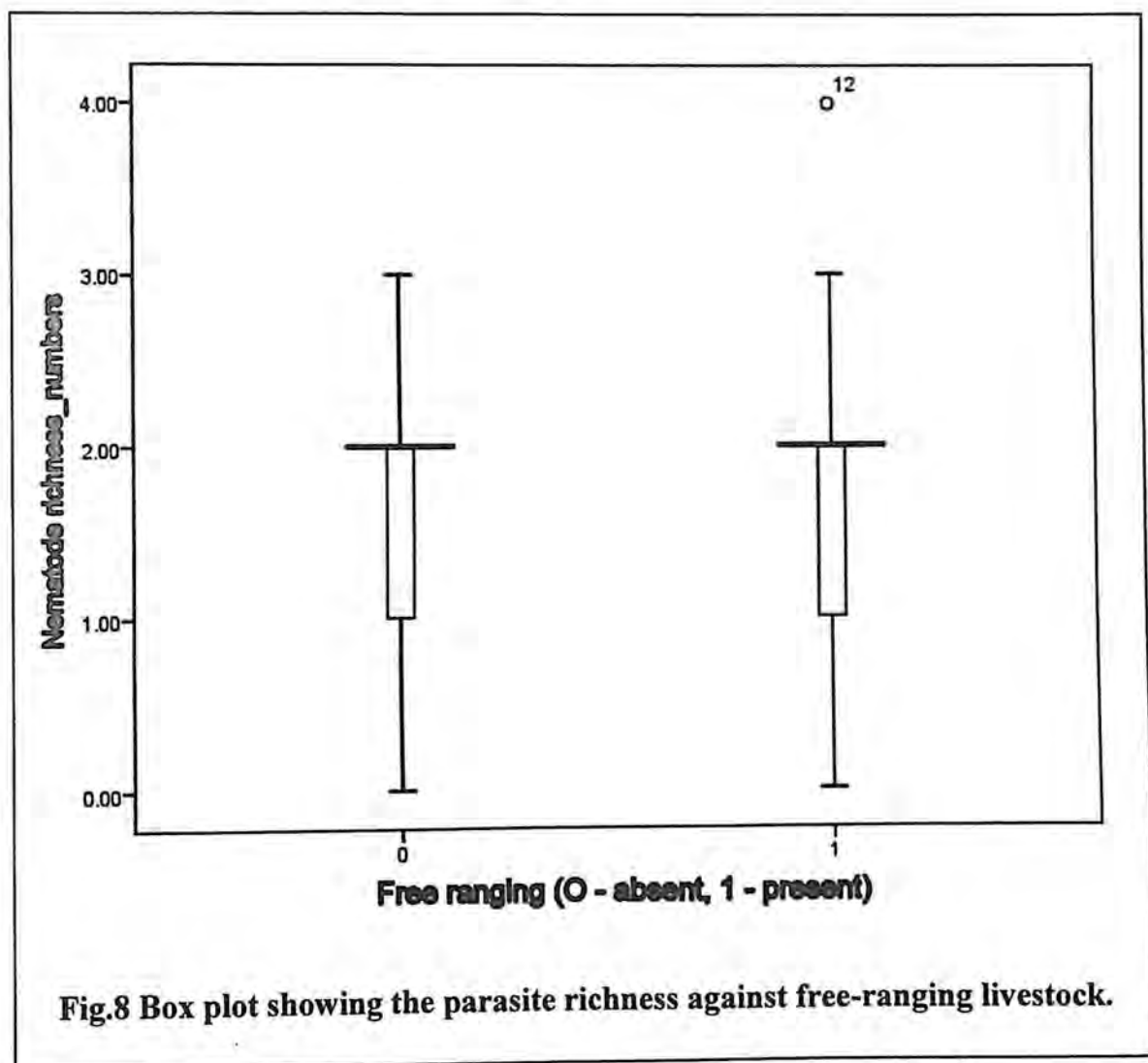
### 3.2 Livestock parasite richness data

The parasites found in the livestock dung samples are:

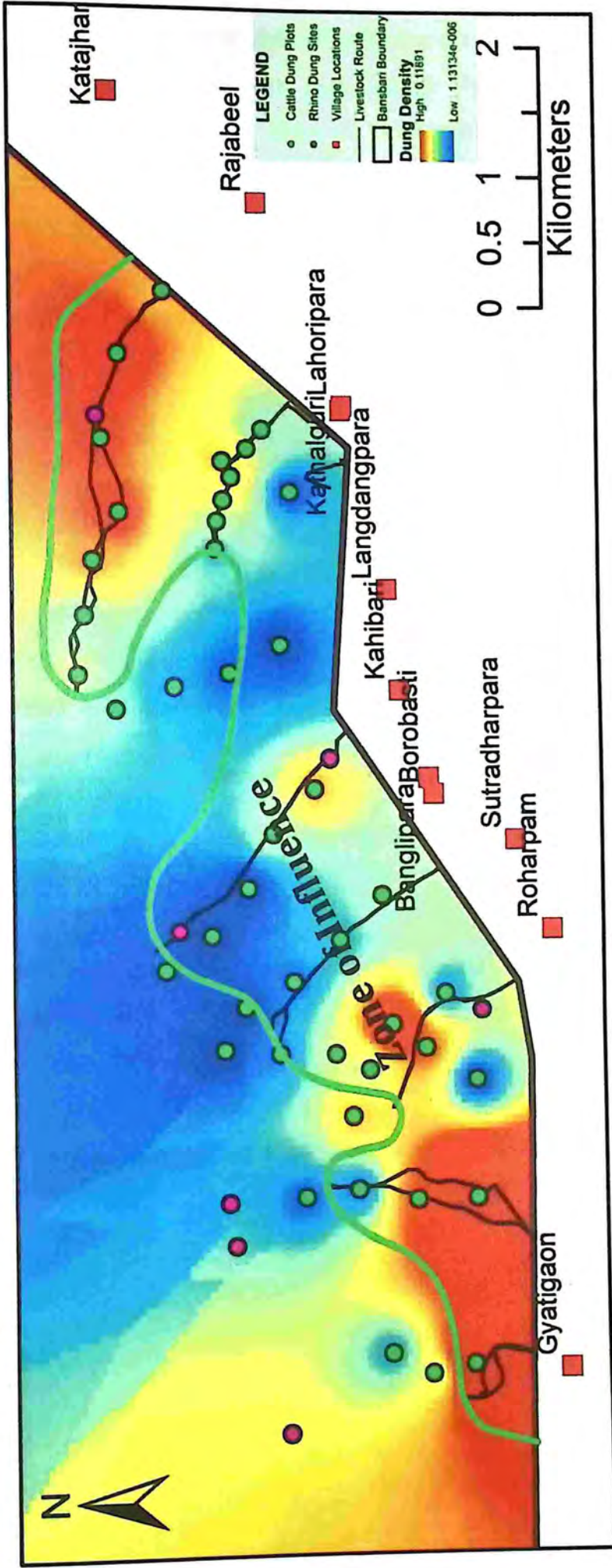
*Haemonchus sp.*, *Paramphistomum sp.*, *Eimeria sp.*, *Toxocara vitulorum*, *Fasciola gigantica*, *Schistosoma sp.*, and *Oesophagostomum sp.* (Fig. 6 & 7).



There is no significant difference in parasite richness between livestock that were primarily free-ranging and those that were primarily stall-fed and occasionally left free ranging (F 0.073, p 0.788) (Fig.8); livestock drinking water from river flowing through the park (F 0.132, p 0.717), livestock procured from market (F 0.453, p 0.503) and livestock which were administered veterinary treatment (F 3.466, p 0.065). This has been shown by plotting in a box-plot where parasite richness has been plotted in Y-axis and free-ranging livestock in X-axis. There is no significant difference in the parasite richness in both the free-ranging (denoted as 1) and non-free ranging livestock (denoted as 0) in the figure below.

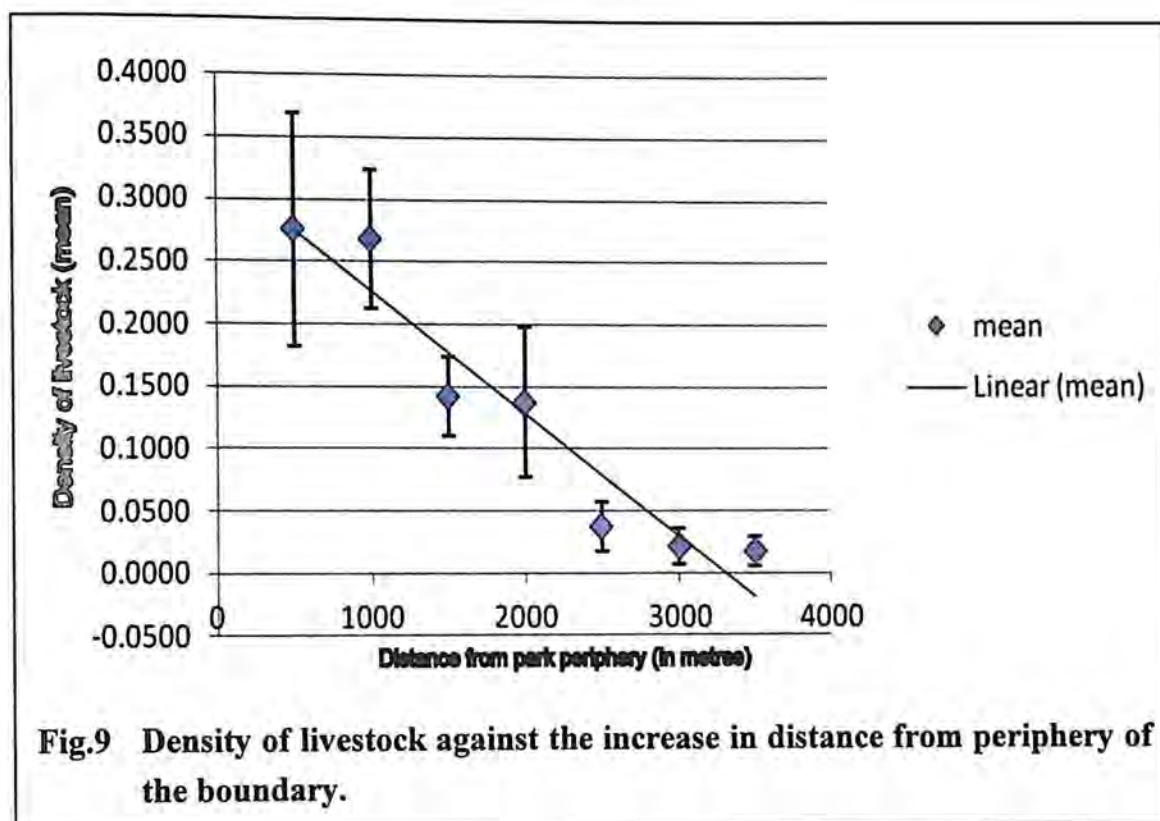


### 3.3 Livestock density inside ZOI

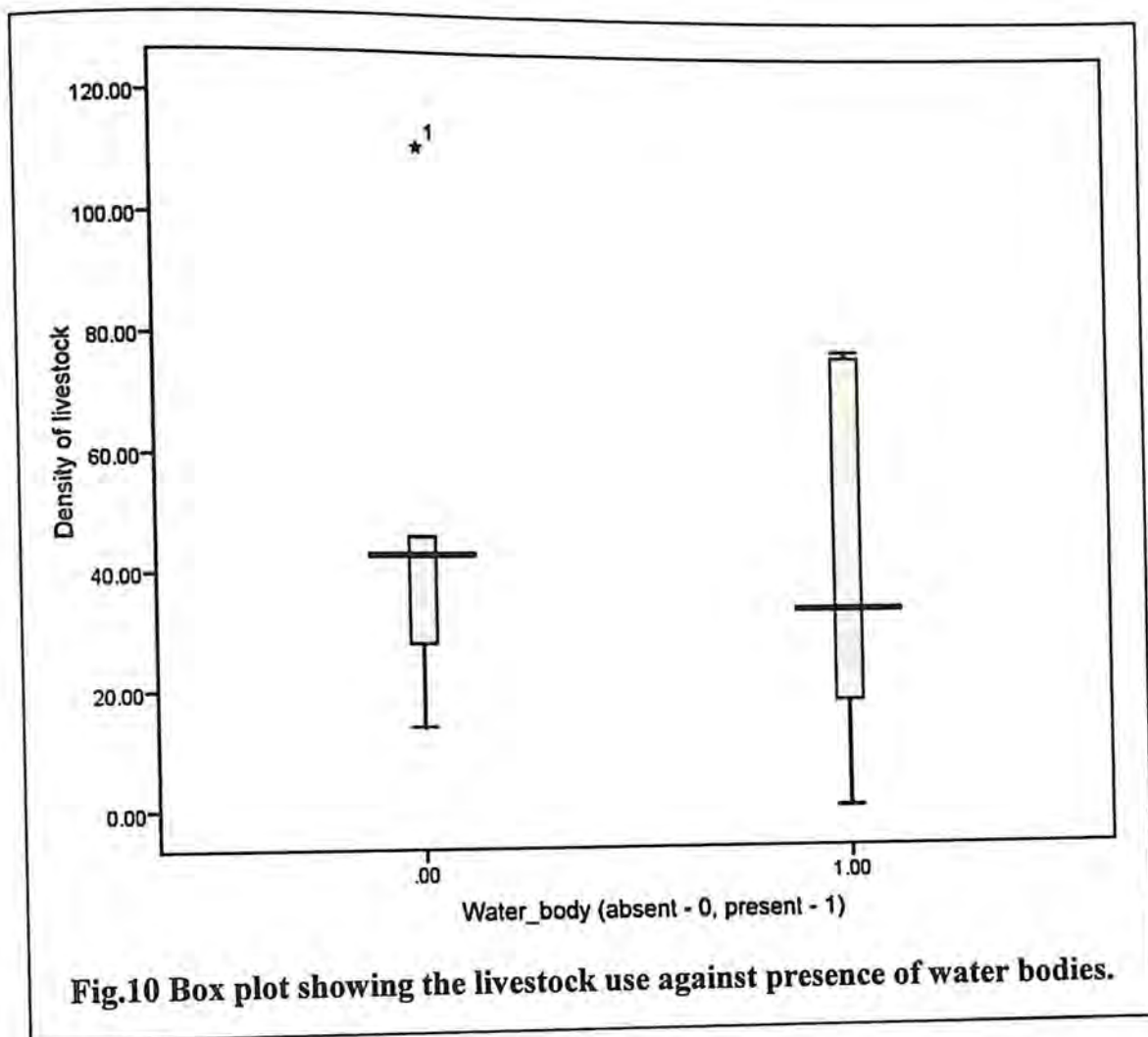


Map 8. Map showing the livestock influence inside ZOI using interpolation technique in GIS.

The density of livestock inside zone of influence (ZOI) decreases with the increase in distance from the periphery of the park (Fig.9). This has been shown in the graph below where mean density of livestock inside the park is plotted in Y-axis and distance from the periphery of the park boundary (in meters) has been plotted in the X-axis.



The density of livestock is more near water bodies inside the zone of influence (p 0.000). The presence of water bodies in an area increases the livestock use of that area. This has been shown by plotting it against a box-plot where the Y-axis denotes the number of livestock inside the park and X-axis denotes the presence (represented as 1) and absence (represented as 0) around water bodies (Fig.10).



This has also been shown by regressing livestock presence (denoted as 1) and absence (denoted as 0) against presence of water bodies (Table 9).

**Table 9. Parameter Estimates of livestock density to presence of water body**

Parameter	B	Std. Error	95% Wald Confidence Interval		Hypothesis Test		
			Lower	Upper	Wald Chi-Square	df	Sig.
(Intercept)	39.667	.4082	38.867	40.467	9440.667	1	.000
[Water_body=.00]	8.733	.6055	7.547	9.920	208.012	1	.000
[Water_body=1.00]	0 <sup>a</sup>	.	.	.	.	.	.
(Scale)	1 <sup>b</sup>						

Dependent Variable: Density

Model: (Intercept), Water\_body

a. Set to zero because this parameter is redundant.

b. Fixed at the displayed value.

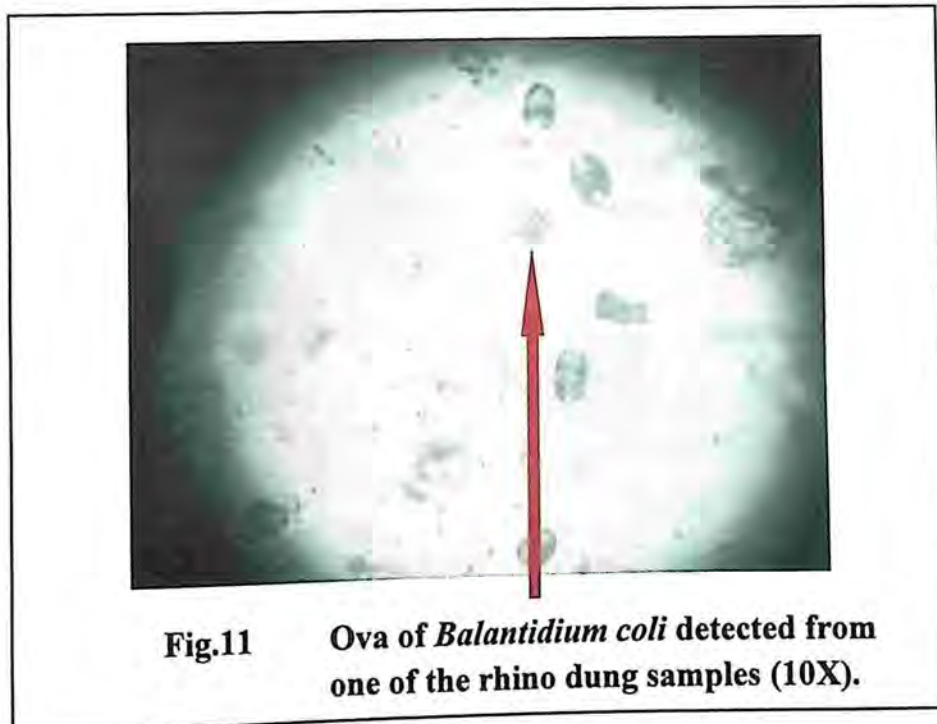
### 3.4 Disease risk from livestock presence

Rhino dung analysis revealed the presence of parasitic eggs/ova of *Paramphistomum* sp. and *Balantidium coli* (heavy load) (Table 10 & Fig. 11).

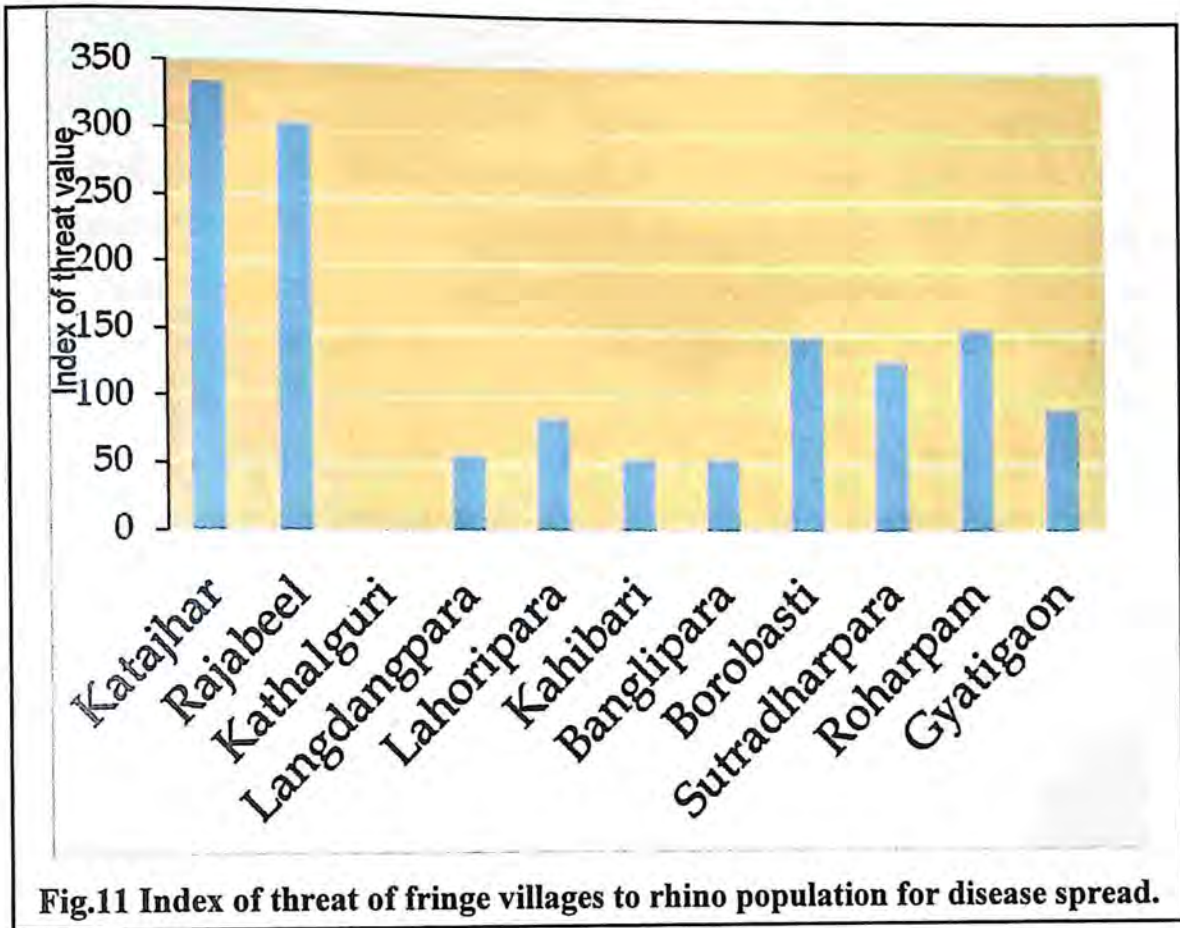
**Table 10. Parasitic load of rhino dung samples analyzed.**

Sl. No.	Sample Code	Parasitic Load (eggs/gram or epg)
1	KBL_1	0
2	CPL	0
3	KBL_2	800
4	R_Boma	1100
5	KBR_1	0
6	EBNK	0
7	PCG	0
8	R_Camp_W	0
9	KBR_2	3700

From the 9 fresh rhino dung samples collected - 4 sites were found to be outside the zone of influence (ZOI). Parasitic load is high in 3 of the samples inside the ZOI, which puts forward the notion that the health statuses of rhinos using these areas is low and are immuno-suppressed (Stark et al. 2009).



The rhino dung samples with high and low livestock density had no significant variation in parasitic load. The reason that can be explained to hold true for the statement is that the sample size of rhino dung sample is low.



The Index of threat for each village to the re-introduced rhinos in Manas NP shows variation in their degree of threat (Fig. 11). The value of the Index of threat has been plotted in Y-axis and the name of villages on X-axis.

These values were regressed against the management practice of livestock and presence of water-body inside the ZOI to observe any significant variation in them. The presence of water body (F 0.090, p 0.771) and free-ranging (F 2.131, p 178) has no significant influence on the index of threat to rhinos in the ZOI.

#### 4. DISCUSSION

This work represents the first structured cross-sectional survey of livestock in fringe villages of Manas National Park (MNP), Assam for disease prevalence and highlights the risk they pose to re-introduced rhino population. The civil unrest in Bodoland with consequent loss in biodiversity in Manas Biosphere Reserve including the extermination of the Greater One-horned rhinoceros (*Rhinoceros unicornis*) from 1989-2006 resulted in a larger proportion of the people devoid of basic facilities and increased dependence on forest resources including heavy reliance on livestock grazing inside the park with regular thoroughfare. The remoteness of the fringe villages with poor amenities has also been one of the factors for lack of veterinary care of non-descript livestock that is reared in the area. Minimal investment is made on these livestock, thereby raising the concerns on disease spread and “spill-over” to wild populations in the vicinity (Palmer et. al 2012).

The present study demonstrated prevalence of variety of infectious and parasitic diseases in livestock populations reared in the fringe villages of Manas National Park. The disease prevalence showed high variation in number of animals affected and parasite species diversity across the villages/hamlets. Interestingly, the disease prevalence showed more of a uniform pattern in the villages with respect to husbandry practices across households. However, strong correlation of brucellosis prevalence (0.614) in livestock procured from market was proved by the study. Wambwa (2005) stressed the need for studies on clinical diseases and that serological investigations should be ensured at key points along markets and slaughter houses. Other infectious diseases accounted for in this study were poorly correlated to livestock procured from market. One possible explanation might be that the market is acting as a medium for transmission of Brucellosis from one affected population to another susceptible population of livestock (Cadmus et. al 2006; Robinson 2003; Unger et. al 2003).

The presence of lotic system such as river which flows through protected areas to human habitation has a significant influence on the prevalence of disease in livestock (Rodwell et. al 2001). The study established high density of livestock usage near

water bodies in Manas National Park, which was also shared by rhinos, indicating disease risk.

In terms of spatial pattern of livestock use, zone on influence by livestock decreased as the distance increased from periphery to the inside of the park. Moreover, the grazing pressure and competition of livestock and grassland species of the park in the periphery of the park was a major point of concern. The presence of livestock in the periphery and their grazing pressure has been documented to negatively affect the structure and composition of the park (Mamo and Bekele 2011).

The study revealed high prevalence for infectious diseases namely **blue tongue**, **tuberculosis** and **brucellosis** among livestock. This has necessitated the need for developing effective management strategies to prevent and control infection spreading into other species including rhinos. Re-introduced rhinos are at constant risk from livestock in fringe villages as rhinos have been sharing common areas with livestock. The major intervention requires includes a well planned disease surveillance and management programme, ensuring proper scientific management of livestock and restricting movement of livestock inside the park through proper checks at entry points. Efforts should be focused on villages showing high Index of threat, viz. Katajhar and Rajabeel, while executing any management interventions including veterinary care. Each of the villages has their own set of determinants which influences the distribution pattern of diseases. Hence, further survey methods should focus on village-specific treatments to bring up practical solutions. Given that the ZOI of livestock in MNP includes home range of rhinos, the rhino population is likely to be under disease risk and conservation efforts needs to incorporate disease perspective for recovery efforts and long-term viability of rhinos in MNP.

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Questionnaire Survey Data Sheet for the project

Questionnaire :	GPS Coordinate :
Date :	Sample Code:
	Investigator's name :

QuestionnaireA. Livestock Owner's identity:

1. Name :	
2. Gender:	<input type="checkbox"/> Male <input type="checkbox"/> Female
3. Age Group:	
4. Village :	-Hamlet :
5. How long have you been living in this village :	<input type="checkbox"/> < 5 years <input type="checkbox"/> > 5 years
6. Marital Status:	<input type="checkbox"/> Married <input type="checkbox"/> Unmarried
7. Community	<input type="checkbox"/> Tribal <input type="checkbox"/> Non-tribal <input type="checkbox"/> Scheduled Caste
8. Education level	<input type="checkbox"/> Matriculation pass <input type="checkbox"/> Under Matriculation
9. If married, family strength	<input type="checkbox"/>
10. Source of income	<input type="checkbox"/>

**B. General Information for Livestock:**

(Use [√] inside the box, answers can be more than one)/fill the answer where applicable

1. Ownership status of land holding	<input type="checkbox"/> Own <input type="checkbox"/> Lease
2. Type of livestock that you have with number of individuals	<input type="checkbox"/> Water buffalo <input type="checkbox"/> Sheep/Goat <input type="checkbox"/> Cattle <input type="checkbox"/> Horse <input type="checkbox"/> Chicken/poultry (please specify the details) <input type="checkbox"/> Any other (provide details)
3. Total no. of livestock	<input type="checkbox"/>
4. Since when are you having/owning livestock?	<input type="checkbox"/> <5 years <input type="checkbox"/> >5 years

**C. Livestock Management:**

(Use [√] inside the box for answer)

1. How are the livestock being managed?	<input type="checkbox"/> Stall-fed <input type="checkbox"/> Free-ranging <input type="checkbox"/> Both stall-fed and free ranging <input type="checkbox"/> Others (please provide details)
2. Feeding source	<input type="checkbox"/> Buying a special feed <input type="checkbox"/> Forage planted by your own <input type="checkbox"/> Let the livestock free to feed by themselves
3. Water source	<input type="checkbox"/> No <input type="checkbox"/> River/stream inside park <input type="checkbox"/> Source inside village <input type="checkbox"/> Same as used for drinking of humans <input type="checkbox"/> Different as to what used for drinking of humans (specify)
4. Where are the livestock procured from?	<input type="checkbox"/> Provide details

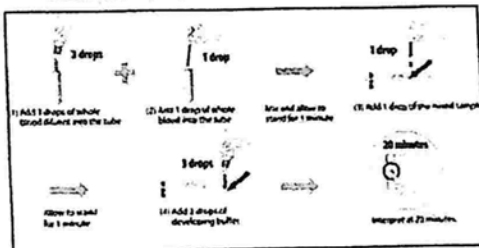
<b>5. When was the last time the livestock showed signs of disease?</b>	<input type="checkbox"/>
<b>6. Is veterinary aid provided to the livestock?</b>	<input type="checkbox"/> Yes <input type="checkbox"/> No
<b>7. What treatment provided to the livestock when sick?</b>	<input type="checkbox"/> Traditional <input type="checkbox"/> Veterinary treatment

## Rapid diagnostic kit manual for bovine tuberculosis (BTB) diagnosis (BIONOTE™)

### [Whole blood specimen]

- 1) Remove the test kit from the foil pouch, and place it on a flat, dry surface.
- 2) Dispense 3 drops of the whole blood diluent into the test tube for the whole blood dilution. Add 1 drop (30µl) of a whole blood sample with the disposable dropper and mix them for 1 minute.
- 3) Add 10µl of the mixed sample with the capillary tube to the sample hole marked "S" on the test device and wait for 1 minute.
- 4) Dispense 3 drops of the developing buffer into the developing buffer hole.
- 5) For the test result, you will see the purple band in the result window of the kit. Interpret test results at 20 minutes. Do not interpret after 30 minutes.

**Caution:** The above result interpreting time is based on reading test results at room temperature of 15 – 30 °C. If your room temperature is not significantly more than 15 °C, the result interpreting time should be properly increased.



### 7. Interpretation of the Test

- 1) A color band will appear in the left section of the result window to show that the test is working properly. This band is the Control line (C).
- 2) The right section of the result window indicates the test results. If another color band appears in the right section of the result window, this band is the Test line (T).
- 3) **Negative:** The presence of only one purple color band within the result window indicates a negative result.

- 4) **Positive:** The presence of two color bands ("T" band and "C" band) within the result window, no matter which band appears first, indicates a positive result.



- 5) **Invalid:** If the purple color band is not visible within the result window after performing the test, the result is considered invalid. The directions may not have been followed correctly or the test may have deteriorated. It is recommended that the specimen be re-tested.



### 8. Limitations of the Test

- 1) Arigen Rapid Bovine TB Test Kit will only indicate the presence of antibodies against *Mycobacterium bovis* in the specimen.
- 2) As with all diagnostic tests, all results must be interpreted together with other clinical information available to the veterinarian.
- 3) If the test result is negative and clinical symptoms persist, additional testing using other clinical methods is recommended. A negative result does not at any time preclude the possibility of *Breucella*.

### 9. Expected Values

Arigen Rapid Bovine TB Test Kit has been compared with a PPD tests. The overall accuracy is greater or equal to 85.5%

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**BIONOTE**

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### I. TECHNICAL BASIS

The kit has been designed to detect antibodies specific for BTM in serum, and a color change will be observed in wells containing positive sera and microcarrier beads coated with BTM. The kit is based on the indirect immunoenzymatic assay technique. The color change is produced by the action of BTM. After adding the sample to the well, if antibodies specific antibodies against the antigen, they will bind to the antigen adsorbed on the microcarrier bead. If the sample does not contain specific antibodies there will not be color change.

If we add a specific monoclonal antibody against the antigen adsorbed to the well (conjugated with peroxidase), it will compete with the antibodies of the sample. If the sample contains specific antibodies, they will not permit the binding of the labeled anti-BTM antibody. If it does not contain specific antibodies the well will bind to the conjugate with the peroxidase. After adding the substrate all over the well, the color change will be produced in absence of antibodies. By adding the substrate (TMB), the color change of the peroxidase will develop a chromogenic reaction.

### II. PRECAUTIONS AND WARNINGS FOR USERS:

1. Read the instructions of use carefully.
2. Bring all reagents to room temperature (20-25°C) prior to use.
3. Do not use instructions or reagents from additional kits.
4. Avoid any contamination of the reagents of the kit.
5. Do not use contaminated glassware, plates and all other components from different kits.
6. These should be kept away from children, or handling under appropriate PPE measures and being handled.

7. Do not pipette by mouth.
8. Use a new tip for each serum sample.
9. For each aliquot of the kit, control positive and negative serum must be tested in a systematic way.
10. Silver solution is a strong acid solution that must be used with protection. In case of accidental contact with skin, wash quickly with water.
11. Substrate must be handled with care, it is very sensitive to light and contamination.

### III. STORAGE OF COMPONENTS

All reagents and plates must be stored at +4°C.

### IV. INFORMATION ABOUT THE WASHING STEPS

The washing steps must be done using an automatic washing machine or a microcarrier washing device suitable for dispensing 100 µl of each well.

After the incubation period, the washing steps must be done following the next instructions:

These are the contents of the plate for 8 samples have over 100 µl of each well. The possible washing of the format from one well to another.

Dispenser is better of 100 µl of washing solution to each well.

1. Wash thoroughly the plate, avoiding the contamination between wells.
2. Turn over the plate vertically to empty the wells.
3. Repeat the washing in each times as is indicated in the instructions of the kit.
4. Prior to using the bottom of the 96-well washing plate, verify that the wash reagent to be added to this plate is ready to use. Do not wash the plate on any other date than freshly washed.
5. After the last step of washing inside the plate turned over an inverted filter paper.

### V. PREPARATION OF REAGENTS

1. Washing solution:  
Dilute one part of the concentrated washing solution provided in the kit with 20 parts of distilled or deionized water (i.e. 10 ml of concentrate and 900 ml of water). Once prepared, this solution remains stable at +4°C.
2. Positive and Negative serum controls:  
Controls must be treated as serum control using 50 µl of each well of 100 µl of volume.

ALL REAGENTS must be stored at +4°C until the moment of use. For each sample, use the following washing solution and use within 24 hours.  
Positive and Negative serum controls: Controls must be ready to use and must be stored during 100 µl of each well.

### VI. PREPARATION OF SAMPLES

1. Serum samples:  
They must be allowed to stand at 22°C in ethanol. The double check for each sample of the assay plate by adding 50 µl of ethanol and 70 µl of sample to each well.

ALL REAGENTS must be stored at +4°C until the moment of use. For each sample, use the following washing solution and use within 24 hours.

### VII. TEST PROCEDURE

1. All reagents must be allowed to come to room temperature before use.
2. Addition of reagents:  
a. Add 50 µl of ethanol to each well. Add 50 µl of each sample to the ethanol.  
b. For control, add 50 µl of a 10 µl ethanol to each well.  
c. Add 100 µl of each sample to the ethanol.  
d. Add 100 µl of positive control and 100 µl of negative control to two wells.  
e. Washed according to the instructions of the kit.

3. Heat the plate and incubate over night (18-24 hours) at room temperature. At the following day, heat the plate at 37°C.
4. Wash 5 times, following the described procedure. At each of wash, 5 wells per well enough slowly, without the substrate.
5. Add 100 µl of conjugate ready to use to each well. Heat the plate and incubate for 30 min. at 37°C.
6. Wash 5 times following the described procedure.
7. Add 100 µl of substrate to each well using the plate for 10 min at room temperature.
8. Add 100 µl of stop solution to each well.
9. Heat the kit of each well at 60°C for 10 min.

### VIII. READING AND RESULT INTERPRETATION

In this kit the sample has been run on duplicate, it has to be considered the mean of both OD values. In the same way, the mean of the values obtained in the kit and of positive and negative control is the correct way to be made.

RESULTS INTERPRETATION (TABLE 1)

Positive Control / Negative Control = 2.25  
Cut Off = 0.55 x Positive Control  
Cut Off (+) = 0.55 x Negative Control  
Cut Off (-) = 0.5 x Negative Control

RESULTS INTERPRETATION (TABLE 1)

Meaning % of sample = 100 - (Cut Off / OD) x 100

Samples with no treatment POSITIVE (ODs are considered specific for BTM), when an OD of 0.55 was higher than the positive cut off (50 µl of conjugate ready).

Samples will be considered NEGATIVE (when not an antibody specific for BTM in the sample) when the OD value is 0.55 or lower or higher than the respective cut off (50 µl of conjugate ready).

Samples with percentages of washing between lower and higher than the respective POSITIVE. It shows that a new sample of the kit is recommended to be analyzed in few weeks.