Spatial and Temporal Overlap of Nilgai (*Boselaphus tragocamelus*) and Cattle (*Bos taurus*) and Its Implications for Parasite Burden and Prevalence

By

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DECLARATION

I hereby declare that the work conducted under the thesis entitled **Spatial and Temporal Overlap of Nilgai (Boselaphus tragocamelus) and Cattle (Bos taurus) and Its Implications for Parasite Burden and Prevalence,** is a record of original and independent research work done by me and subsequently submitted for the award of the degree of **Master's in Wildlife Science** at the **Academy of Scientific and Innovative Research**. This research work has been carried out under the guidance and supervision of Dr. Lallianpuii Kawlni, Scientist D, and co-supervision of Dr. Vishnupriya Kolipakam, Scientist D, and Dr. Ayan Sadhu, Research Scientist, NTCA of Wildlife Institute of India, Dehradun. The work has not formed the basis for the award of any other degree, diploma, or any other qualification. I also declare that the thesis embodies my work, analysis, observation, understanding, and the particulars given in it are true to the best of my knowledge.

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This is to certify that the thesis by **Surya Pratap Singh** entitled **Spatial and Temporal Overlap of Nilgai (*Boselaphus tragocamelus*) and Cattle (*Bos taurus*) and Its Implications for Parasite Burden and Prevalence** is an original and independent research work submitted to the **Academy of Scientific and Innovative Research**, for the award of the degree of **Master's in Wildlife Science**. **Surya Pratap Singh** has put one semester of research work embodied in this thesis under my guidance and supervision. The work presented in this thesis has not been submitted to any other University or Institute for the award of any degree, diploma, or distinction.

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EXECUTIVE SUMMARY

Humans have depended on domesticated animals and plants since the origin of agriculture, and forests and grasslands have provided essential resources such as grazing and forest products. But there is greater overlap today between livestock and wildlife as a result of greater deforestation and growing human activity, and this has implications for the transmission of disease at these interfaces.

In this dissertation, the impacts of spatial overlap, environmental variables, and animal behavior are investigated for their influence on patterns and drivers of gastrointestinal parasite load in nilgai (Boselaphus tragocamelus) and cattle, a commensal wild ungulate of the Indian peninsula. During December 2024 to March 2025, the study was conducted in the Darra enclosure of Mukundra Hill National Park.

In addition to spatial information regarding animal locations with respect to villages, water points, and livestock distribution, field measurements were collected on parasitic burden, which was expressed as eggs per gram (EPG) in fecal samples. The research attempted to determine the impacts of spatial overlap among livestock and wildlife and proximity to these attributes on parasite intensity and prevalence.

Statistical tests revealed no substantial relationship between the parasitic load of cattle and their proximity to villages or water points, contrary to preliminary hypotheses. All individuals sampled carried high EPG values consistently, revealing an overall parasite burden. Widespread parasite exposure regardless of spatial gradients due to common grazing, minimal pasture rotation, and homogenous environmental conditions are most likely the primary reasons for such a pattern.

The research explored whether temporal and spatial coincidences with livestock, in this case cattle, can be channels of parasite transmission for nilgai. The findings indicate that the impact of spatial proximity can be overcome by aggregating at shared resources and chronic exposure to soiled environments and that it consistently yields high infection rates in domestic and wild ungulates.

These results underscore the importance of considering environmental context, grazing management, and animal behavior when designing parasite control strategies. Instead of simply considering physical distance to villages or water sources, interventions taking into account pasture management and resource allocation may be more effective. The research presents valuable information for the prevention of disease, management of wildlife, and conservation of India's domestic and wild ungulates.

CHAPTER 1: INTRODUCTION

1.1 LITERATURE REVIEW

Parasite ecology studies the interactions between hosts and parasites as a dynamic system. Parasitism is a biological interaction where one organism lives off the other organism, in or on the body of the other organism (host), damaging the host's body structure, metabolism, or both. (Rózsa & Garay, 2023). Host-parasite ecosystems evolve simultaneously, a process known as coevolution. Host-parasite ecosystems coevolve through reciprocal selection pressures-adaptations in one species drive counter-adaptations in the other, forming a coevolutionary feedback loop (Buckingham & Ashby, 2022). The impact of parasites can be projected at the fine-scale level, which is from an organism to the population level. Even when not causing acute mortality, parasites may cryptically alter host behaviour, physiology, and reproductive success, which can gradually influence population size and structure, and disrupt trophic interactions, food webs, and biodiversity These interactions imply that parasites are integrated into an ecosystem and shape community and ecosystem structure (Preston & Johnson, 2010).

Parasites are one of the many factors that can induce local declines and extinctions in vulnerable wild animal populations. This gets more compelling since the current anthropogenic period is taking a toll on environmental health. Human activities, such as land use change and habitat fragmentation, are known to alter wildlife diversity. Since host and parasite diversities are strongly connected, this increases the risk of emerging infectious diseases (Chakraborty et al., 2019). Anthropogenic activities increase stress on hosts, making them more susceptible to parasitic infections. These stressors can weaken the host's immune system or physiological capacity, reducing their ability to resist or combat parasite invasion (Zuzarte-Luís & Mota, 2018).

Parasitic disease remains a key challenge in the field of wildlife management, especially for community reserves and wildlife sanctuaries, where shared spaces serve as transmission pathways for various parasites. Transmission dynamics of various parasites are strongly associated with environmental characteristics and the spatial patterns of host populations. Environmental characteristics and spatial host distribution significantly shape transmission dynamics, with risk factors including contaminated waterholes, grazing areas, village peripheries, carcass sites, and dumping grounds. Understanding the spatial-temporal factors influencing parasite load in managed landscapes is essential for designing strategies that minimize infection risk and enhance wildlife health. This dissertation aims to address this gap through systematic field sampling and spatial analysis, aiming to inform evidence-based control and management strategies.

1.2 PARASITES

According to the Centers for Disease Control and Prevention (CDC, 2024), a parasite is an organism that lives in, on, or with another organism (the host) and benefits at the host's expense, often causing harm as it uses the host’s body for nourishment and shelter. The host can be a plant or animal species. While they don't always kill the host, parasites can cause various health problems and diseases (CDC, 2024). Parasites rely on the host for their transmission to the next potential host(s), where they can continue multiplying and spreading. The two main groups of parasites affecting animals and humans are helminths and protozoa (CDC, 2024).

Helminths (Worms) are a general term for a parasitic worm. The helminths include the Platyhelminthes, or flatworms (flukes and tapeworms), and the Nematoda, or roundworms. All helminths are relatively large (> 1 mm long); some are very large (> 1 m long) (W. Akelin, 1996). Platyhelminthes (further divided into classes like Trematoda and Cestoda) and

Nemathelminthes (containing the classes Nematoda, Nematomorpha, and Acanthocephala) (Soulsby, 1968).

Nematodes (roundworms) are members of the phylum Nematoda. They possess long, thin, unsegmented, tube-like bodies with a fluid-filled internal cavity (pseudocoelom) and have a longitudinal digestive tract (Australian Society for Parasitology, n.d.). The members of most nematodes are free-living and inhabit soil and fresh and salt water, as well as other environments. The majority of parasitic nematodes have evolved to develop a highly specific biological dependence on a particular host and are not capable of survival in any other host (Katz et al., 1989).

Cestodes (tapeworms) belong to the class Cestoidea, phylum Platyhelminthes, and include parasitic tapeworms. They have long, segmented bodies. The adults of this class are all parasitic and host in the intestines of mammals (Katz et al., 1989). They do not have a gut, and all nutrients are taken up through the tegument. They are acoelomates, lacking a body cavity, and are flattened to facilitate perfusion to all tissues (Australian Society for Parasitology).

Trematodes (flukes) belong to the class Trematoda, phylum Platyhelminthes, and are divided into two orders, Monogenea and Digenea, both of which consist of obligate parasites. They have small, flat, leaf-like bodies with no body cavity (acoelomate) (Katz et al., 1989).

1.3 TRANSMISSION AND LIFE CYCLE

Gastrointestinal parasites are transmitted through eating contaminated food, drinking water, or ingestion of soil containing larvae, cysts, or eggs (Bundy et al., 2018). Parasites like hookworms can penetrate directly into the skin of the host. Their life cycles can be direct transmission or indirect transmission (Pritt, 2015). Direct transmission is when the host ingests the infective stages directly from the contaminated environment. Indirect transmission occurs when the parasite life cycle involves the intermediate hosts (e.g., snails, ticks, mites, etc.) for its development before infecting the definitive host (Greenwood, 2012).

1.4 DISEASES AND THEIR IMPACT ON POPULATION

Pathogens are an intrinsic part of the biological diversity of a healthy ecosystem that are limited by the natural barriers like environmental conditions or the distribution and behaviour of their hosts and vectors (Peterson & Ferro, 2012). Even though the occurrence of disease in nature is a natural regulatory process, there is an increasing trend toward the appearance of novel or introduced diseases. When combined with other stressors on habitats and populations, particularly fragmented populations, disease in wildlife may present a serious conservation concern (Tazerji et al., 2022). Diseases can be the fundamental natural forces that shape animal populations by influencing survival, reproduction, and overall fitness when they occur naturally, but they can pose a threat to natural diversity when introduced. One such disease is chytrid fungus (*Batrachochytrium dendrobatidis*), which has caused havoc in the amphibian community, affecting 700 species all across the globe. One study (Scheele et al., 2019) found that chytrid fungus (*Batrachochytrium dendrobatidis*) has contributed to the decline of at least 501 amphibian species, that is about 6.5 percent of all amphibian species described so far. White-nose syndrome, caused by the fungus *Geomyces destructans*, is taking the lives of cave-roosting bats across North America, and the mortality caused by sylvatic plague, wiping out the colonies of black-tailed prairie dogs (*Cynomys ludovicianus*) (Roth, 2019; Pearse & Swift, 2006).

The transmissible cancer is causing declines of Tasmanian devils (*Sarcophilus harrisii*) (Flacke et al., 2013). In 2006, a significant decline in the largest African wild dog (*Lycaon pictus)* pack in KwaZulu-Natal (KZN), South Africa, was observed. The pack, originally consisting of 26 individuals, declined to a single animal. The most likely cause for this drastic reduction was a disease caused by the rabies virus. The study also highlighted that canine distemper virus (CDV), canine parvovirus (CPV), and rabies are potential threats to African wild dog (*Lycaon pictus)* conservation in KZN (Paulson, McCullough, & Cheville, 1998). Bacterial diseases can also exert significant pressure on wildlife populations, as exemplified by the persistent presence of brucellosis in bison (*Bison bison)* and elk (*Cervus canadensis*) within Yellowstone National Park, USA, which poses ongoing challenges for wildlife management and can affect reproductive success and population dynamics (Cheville, 1999; Rhyan & Spraker, 1987). Some pathogens can also be transmitted among conspecifics, other wildlife species, domestic animals, and humans, posing a risk to humans' and animals' health and resulting in significant economic impact (Woodroffe et al., 2009). The role of bacterial pathogens in wildlife population dynamics is evident in the case of bovine tuberculosis (*Mycobacterium bovis*) in European badgers (*Meles meles*) in the United Kingdom (Hampson et al., 2011). Sporadic but often significant mortality events in wild ungulate populations can be attributed to bacterial diseases such as anthrax (*Bacillus anthracis*), with outbreaks occurring in various regions, including Africa, North America, and Asia, impacting local population sizes and potentially having broader ecological consequences (Olano, Weller, Guerrant, & Walker, 2011). Some parasites have hitchhike on host death for transmission. For instance, 'obligate killer' parasites such as chytrid infections in Daphnia, fungal parasites of ants, and parasitoid wasps that can only spread or persist following the eventual death of the infected host (Lee et al., 2025).

Unlike microparasitic pathogens such as viruses, bacteria, protozoa, and fungi, helminths—being macroparasites—typically do not cause abrupt death in their hosts. This is largely because, unlike other pathogens, helminths do not proliferate within their hosts; instead, individual worms grow, molt, mature, and then produce offspring that are expelled to infect new individuals (Lee et al., 2025). Consequently, the worm burden in a host, and often the severity of infection, is directly associated with the uptake of infective microorganisms from the environment. Compared to the rapid development and multiplication of microparasites, the development of helminths is a slower process, leading to diseases that are often slow in onset and chronic in nature. While many helminth infections are well-tolerated and asymptomatic, even subclinical infections can result in significant loss of condition in affected hosts. Conversely, some helminths can cause serious clinical diseases characterized by high morbidity and mortality (Woodbury et al., 2012). Notable wildlife disease events, such as the outbreaks of sarcoptic mange in the reintroduced red fox (*Vulpes vulpes*) population of South Korea between 2019 and 2024, resulted in 12 individuals being found dead (Otero-Abad & Torgerson, 2013). The roundworm (*Baylisascaris schroederi)* is one of the major causes of mortality in wild giant pandas (*Ailuropoda melanoleuca*) (Moss et al., 2013).

The discovery of the parasite *Toxocara vitulorum* in bison calves in Manitoba, Canada, is a case of the introduction of the parasite by exotic animals. *Toxocara vitulorum sp.* is more commonly found in the small intestines of bovid calves living in tropical and subtropical regions of the world. This is the first time that *Toxocara vitulorum* has been reported from hosts in Canada (Wilber, Briggs, & Johnson, 2020). Lungworms do not usually have a pathogenic effect on the host; however, when associated with bacteria of the genus *Pasteurella*, they cause lungworm-pneumonia complex in bighorn sheep (*Ovis canadensis*). Mortality resulting from bronchopneumonia has limited the relative abundance of bighorn sheep (*Ovis canadensis)* across North America (Johnson et al., 2006).

In wolves, *A. caninum* infection has been associated with iron deficiency anemia in pups (Bordes & Morand, 2011). In Florida, a young cougar (*Puma concolor*) was found markedly anemic due to *A. pluridentatum* infection (Dunbar et al., 1994). Echinococcosis, also known as hydatid disease, is a parasitic infection in canids (dogs, foxes, coyotes, etc.) caused by the tapeworms *Echinococcus granulosus* and *Echinococcus multilocularis*. These tapeworms live in the small intestine of canids, and the adult parasites release eggs that are shed in the canid's feces (Zibaei et al., 2020). Heavy infestations of Echinococcosis in hooved mammals may be associated with poor body condition, and large numbers of hydatid cysts in the lungs may lead to respiratory distress and exercise intolerance. In moose, it may reduce the lung capacity and stamina, making them more vulnerable to predation (Dunbar, McLaughlin, Murphy, & Cunningham, 1994). These prey species are then the transmission route for parasites to reach a new host, which affects the predators as well. Strongyle nematode parasites that persist endemically in wild sheep populations decrease body weight and fecundity in adult sheep and increase overwinter mortality in juveniles (Delong, Orr, Jenkinson, & Lyons, 2009). A study conducted by Barone, Wit, Hoberg, Gilleard, and Zarlenga (2020) showed that hookworm infection in Northern fur seals (*Callorhinus ursinus*) retards individual growth.

1.5 PARASITIC DISEASES IN RUMINANTS

The study by Modabbernia, Meshgi, and Eslami (2021) found that helminthiasis in wild ruminants (*Ovis orientalis, Capra aegagrus, and Gazella subgutturosa)* affects various organs, including the gastrointestinal tract, heart, liver, and lungs, and found that Intestinal nematodes were Marshallagia marshalli**,** Ostertagia ostertagi**,** Skrjabinema ovis**,** Nematodirus oiratianus and longispiculata**,** Trichuris ovis**,** and Trichuris discolor**.** Lungworms—Protostrongylus rufescens, Cystocaulus ocreatus; Cestodes—Moniezia, Helicometra, Giardia, Avitellina centripunctata, Stilesia globipunctata (Modabbernia, Meshgi, & Eslami, 2021).

Jolles and colleagues investigated microparasite helminth coinfections and demonstrated that African buffaloes (Syncerus caffer) coinfected with *Mycobacterium bovis* and gastrointestinal nematodes had a reduced lifespan and a worsened body condition compared to African buffaloes infected only by bovine tuberculosis or only by digestive worms. Interestingly, nematodes facilitated tuberculosis invasion by inducing immunosuppression effects (Bordes, F., & Morand, S., 2011)

Wild ruminants in North America were documented to harbour a total of 54 species in 16 genera of nematodes, primarily of three superfamilies: Ancylostomatoidea, Strongyloidea, and Trichostrongyloidea, with the latter being the most taxonomically diverse and numerically dominant, as well as the most important in terms of pathogenic potential. The host range includes six species of wild bovids—such as pronghorn (*Antilocapra americana*), bison (*Bison bison*), mountain goat (*Oreamnos americanus*), bighorn sheep (*Ovis canadensis*), Dall's sheep (*Ovis dalli*), and muskox (*Ovibos moschatus*)—and five species of wild cervids, including moose (*Alces alces*), elk (*Cervus elaphus*), mule deer (*Odocoileus hemionus*), white-tailed deer (*O. virginianus*), and caribou (*Rangifer tarandus*). Additionally, domestic ruminants such as cattle, sheep, and llamas are important hosts, often sharing parasite species with their wild counterparts (Jenkins, Hoberg, Rosenthal, & Gilleard, 2020).

The genus *Monodontus*, particularly *Monodontus louisianensis*, is notable as the only hookworm reported from wild cervids, primarily infecting the small intestine of white-tailed deer (*Odocoileus virginianus*) in the southern United States. The genus *Eucyathostomum* is also present in these hosts. However, the most significant diversity is observed within the superfamily *Trichostrongyloidea*, which includes genera such as *Ostertagia*, *Teladorsagia*, *Marshallagia*, and *Haemonchus*. (Jenkins et al., 2020).

Tichostrongyloid nematodes have been diagnosed in various parts of the gastrointestinal tract, like the abomasum, small intestine, and large intestine; some species are capable of inhabiting more than one part of the host body (Hoberg, Kocan, & Rickard, 2001).

Taenia saginata (beef tapeworm) has been reported from cattle (*Bos taurus*), water buffalo (*Bubalus bubalis*), and reindeer (*Rangifer tarandus*). It uses these species as intermediate hosts, and humans as the definitive host. It is cosmopolitan but most common in regions with poor sanitation and where raw or undercooked beef is eaten, such as parts of Latin America, Asia, and sub-Saharan Africa.

Taenia hydatigena, *Taenia multiceps*, and *Taenia ovis* life cycles involve sheep (*Ovis aries*), goats (*Capra hircus*), cattle (*Bos taurus*), pigs (*Sus scrofa domesticus*), wild boar (*Sus scrofa*), reindeer (*Rangifer tarandus*), cervids (family *Cervidae*), and antelopes (various species in the family *Bovidae*) serving as intermediate hosts, with dogs (*Canis familiaris*), wolves (*Canis lupus*), and other canids as definitive hosts (Jones & Pybus, 2001).*Taenia krabbei* is found in wild canids—wolves (*Canis lupus*), Arctic foxes (*Vulpes lagopus*), and coyotes (*Canis latrans*)—as definitive hosts, and in cervids (family *Cervidae*), muskoxen (*Ovibos moschatus*), and mouflon (*Ovis orientalis musimon*) as intermediate hosts, mainly in northern temperate regions.

Echinococcus genus parasites affect wild and domestic ruminants and herbivores worldwide. For example, *Echinococcus granulosus sensu lato* (causing cystic echinococcosis) has been reported from wild goats (*Capra aegagrus*), cattle (*Bos taurus*), yaks (*Bos grunniens*), horses (*Equus ferus caballus*), camels (*Camelus dromedarius* and *Camelus bactrianus*), chamois (*Rupicapra rupicapra*), deer (family *Cervidae*), antelope (various species in family *Bovidae*), and others. It has been reported in wild ruminants—such as deer (family *Cervidae*), antelopes (*various species in family Bovidae*), and other wild bovids—across Europe, Africa, Asia, Australia, and the Americas. The infected individual develops large cysts in the lungs or liver. Studies in North America have shown that moose with lung cysts are more likely to be preyed upon by predators. In Australia, a similar result was seen in macropod marsupials infected with *E. granulosus*, where lung cysts hinder their ability to escape Dingos (*Canis lupus dingo*). The presence and transmission of Echinococcus in wildlife are influenced by both natural predator-prey cycles and spillover from domestic animal cycles, with human activities and environmental changes playing a role in their distribution and prevalence (Romig & Wassermann, 2024). The natural infection of *Fasciola* was recorded from free-ranging chital (*Axis axis*), blackbucks (*Antilope cervicapra*), wild boar (*Sus scrofa*), and gaur (*Bos gaurus*). Paramphistomes are helminths found in the forestomach of ruminants without an obvious pathogenic effect. The pathogenic effect lies within the immature ones that attach to the duodenal mucosa. Several species of amphistomosis, *Cotylophoron cotylophorum*, *Paramphistomum cervi*, and *Gastrothylax crumenifer*, have been recorded in gaur (*Bos gaurus*), sambar (*Rusa unicolor*), chital (*Axis axis*), blackbuck (*Antilope cervicapra*), nilgai (*Boselaphus tragocamelus*), and swamp deer (*Rucervus duvaucelii*) (Arora, B. N. *Indian wildlife disease and diagnosis*). Most of the major *Schistosoma* species that are diagnosed are from African livestock. In the Asian region, they are recorded from pigs (*Sus scrofa*), dogs (*Canis lupus familiaris*), and captive elephants (*Elephas maximus*). Echinostomids have been unrecorded in wild ruminants; most of the wild records are from civets (*Viverricula indica*) (Amrithras et al., 1999) and honey badgers (*Mellivora capensis*) (Srivastava, 1964). *Paragonimus* was recovered from gray mongoose (*Herpestes edwardsii*) (Cobbold, 1859) and civet (*Viverricula indica*) (Gulati, 1926); there are no records in ruminants. The death of a gibbon (*Hylobates spp.*) with chronic colitis was caused by an overwhelming infection with *Trichuris* and oxyurid parasites (cited by Toft, J.D., 1982, Vet. Path. 19(7): 69). The hosts of *Trichuris* are blackbuck (*Antilope cervicapra*), chital (*Axis axis*), sambar (*Rusa unicolor*), and wild pig (*Sus scrofa*). The fecal samples from rhinoceroses (*Rhinoceros unicornis*) and elephants (*Elephas maximus*) often show low *Strongyle* sp. infestation. Necropsy of one 8-month-old female rhino (*Rhinoceros unicornis*) calf that died in Dudhwa National Park had shown the presence of a large number of tapeworms, *Thysanosoma actinoides*, in its intestine. There were also a few roundworms identified as *Strongylus edentatus*. Mixed infections of *Trichostrongylus columbriformis*, *Haemonchus contortus*, and *Bunostomum trigocephalum* were encountered in wild blackbuck (*Antilope cervicapra*) (Pande et al., 1972). Species of *Trichostrongylus*, *Haemonchus*, *Ostertagia*, *Nematodirus*, and *Cooperia* infect wild ruminants (Arora et al., 1985). *Haemonchus contortus* was found in the markhor (*Capra falconeri*). The parasites were detected in the abomasum of blackbuck (*Antilope cervicapra*) (Patnaik, 1964; Sadana et al., 1980). Earlier, *Haemonchus boispinosus* (Khera, 1954) and *Haemonchus cervinus* (Baylis and Daubney, 1922) were reported in deer (*Axis axis*).

Numerous specimens of *Ashworthius* from the abomasum of two nilgais (*Boselaphus tragocamelus*) (Singh, 1962) were identified as *A. perrilli* (Chauhan et al., 1972). *Ashworthius mertinagliae* Ortepp, 1933 was recovered from the gut of a nilgai (*Boselaphus tragocamelus*) necropsied at Gir Forest, Gujarat (Hiregoudar, 1976). Infestations with members of the genus *Strongyloides* have frequently been reported in elephants (*Elephas maximus*). No outbreak of strongyloidosis in wild animals so far has been documented. The available literature shows that ascarids have more frequently been discovered from wild boar (*Sus scrofa*), chital (*Axis axis*), and mostly carnivores than from other mammalian members (Katz, Despommier, & Gwadz, *Parasitic Diseases*). *Oesophagostomum* sp. has been reported in wild artiodactylids such as hog deer (*Axis porcinus*), chital (*Axis axis*), and nilgai (*Boselaphus tragocamelus*).

1.6 LIVESTOCK-WILDLIFE OVERLAP AND TRANSMISSION DYNAMICS

India has witnessed a consistent and notable increase in its livestock population in the past ten years. Agriculture censuses of the years 2007, 2012, and 2019 reported 199, 191, and 536.76 million cattle, respectively. The result of the 2019 census reflects a 4.6% increase over the previous census in 2012. The rise in livestock population can be seen across major livestock species such as cattle, buffalo, sheep, and goats. Among many factors, the primary factors that are responsible for the increase in livestock populations are rising human populations, urbanization, and income growth, especially in developing countries. The increase in human population, together with globalization, has also influenced the standard of living in developed countries. This is resulting in the rapid growth of farm animals worldwide and also in the increased impact of livestock farming systems (Thornton, 2010; Statista, n.d). This continuous rise in the livestock population has directly led to a rise in fodder demands across the country (Singh et al., 2022; Hilli et al., 2023). Recent assessments done by the Department of Agriculture and Farmers Welfare, 2023, indicate that the cumulative requirement for feed and fodder is immense. The estimated national requirement is about 597.67 million tons of feed and fodder annually. The demand is highest in states with large livestock populations, such as Uttar Pradesh and Rajasthan. As per the 20th Livestock Census, rural areas hold the most number of livestock, which is around 514.11 million, 95.78% of the total livestock population. With the livestock numbers standing at more than 536 million, the resulting demands have intensified the competition for land and resources between food and fodder production. (Singh et al., 2022; Hilli et al., 2023).

As the available agricultural land becomes insufficient to support both crop and fodder needs, many rural households increasingly rely on open-access resources such as forests and common grasslands to graze their animals and collect fodder (Singh, 2003).

This increasing strain has contributed to the encroachment and degradation of both forests and grasslands. The March 2024 government survey says that over 13,000 sq km of forest land across 25 states and union territories is under encroachment; that is nearly double the figure of previous years (Khan et al., 2022), and much of this encroachment has been done by rural populations in need of new grazing grounds or agricultural plots to meet the rising fodder and food demands. States like Madhya Pradesh and Assam are particularly affected by encroachment, occurring not only in reserve forests but also in protected areas and wildlife sanctuaries. (Khan et al., 2022). India lost 31% (about 5.65 million hectares) of its grassland area in just a decade (2005–2015), largely due to overgrazing, poor management, and especially the conversion of pastures into cropland. Looking at the current situation of food and fodder demands, the agricultural land is becoming insufficient to satisfy both crop and fodder needs. Thus, pushing rural households' dependency on open-access resources such as forests and common grasslands to graze their animals and collect fodder (Singh, 2003).

Rural communities hold over 95% of India’s livestock. They have direct, more frequent, and prolonged interactions with wildlife. (Maleko et al., 2012). These interactions are usually driven by grazing resources. Areas like arid and semi-arid landscapes have a huge scarcity of resources during summers, which increases the congregation of both wild and domestic herbivores (Jori et al., 2021), thus increasing the opportunities for direct and indirect transmission of various pathogens, including bacteria, viruses, and parasites, thus facilitating the spillover and circulation of diseases (Varela-Castro et al., 2021).

Rural communities hold over 95% of India's livestock. They have direct, more frequent, and prolonged interactions with wildlife (Maleko et al., 2012). Anthropogenic wildlife encounters have disrupted the integrity of the ecosystem by making way for plastics into the ecosystem food chain in the form of microplastics. Once these plastics are ingested by livestock or wildlife, they are integrated into the food chain system in the form of microplastics, where they can accumulate and pose health risks.

These interactions are usually driven by grazing resources; areas like arid and semi-arid landscapes have a huge scarcity of resources during summers, which increases congregation of both wild and domestic herbivores and limited water and fodder sources (Jori et al., 2021), thus increasing the opportunities for direct and indirect transmission of various pathogens, including bacteria, viruses, and parasites (Varela-Castro et al., 2021).

The parasitic infections can impact the health, survival, and reproductive success of the host, especially helminths and other gastrointestinal (GI) parasites. Prolonged parasitic infection impacts the development rates, reduces reproductive outcomes, weakens immunity, and, in extreme situations, could result in the death of the host. These are just a few of the negative consequences that GI parasites can cause in both domestic and wild animals. Infections cause significant financial losses to farmers because they reduce livestock productivity and increase veterinary expenses.

Spatial-temporal activity overlap between the livestock and wildlife can significantly influence the parasitic transmission in wild animals. The degree of overlap may also determine the intensity and prevalence of infections in the wild population. Understanding spatial-temporal dynamics is crucial for understanding disease dynamics in wildlife and hotspot areas.

60-80% of newly emerging infectious diseases are zoonotic, with at least 70% originating from the interface between wildlife, livestock, and humans. The livestock–wildlife interface enables the transmission of diseases from wildlife reservoirs to domestic animals and then to humans, or vice versa. Notable zoonotic pathogens include HIV (from bushmeat consumption), Ebola virus, Cyclospora, Escherichia coli O157:H7, rabies, cowpox virus, Lyme disease (*Borrelia burgdorferi*), Nipah, and Hendra viruses in fruit bats, as well as foodborne and antibiotic-resistant pathogens. In urban areas, rodents, bats, and synanthropic mammals (such as foxes and raccoons) are important urban wildlife reservoirs for zoonoses like plague, leptospirosis, and hantavirus (Zubair et al., 2024; Hassell et al., 2017).

Urbanization is expanding rapidly and transforming landscapes and wildlife communities leading, to both a reduction in overall parasite diversity and an increase in transmission of certain pathogens among urban-adapted hosts, pathogens such as *Borrelia burgdorferi* (causing Lyme disease), which sees higher prevalence in ticks and their primary reservoir, the white-footed mouse, in fragmented suburban landscapes of North America; West Nile virus (WNV), which is more common in urban songbirds and can spill over to humans; and *Baylisascaris procyonis* (raccoon roundworm), which increases in prevalence among urban raccoon populations due to resource clumping. The zoonotic Parasite includes tapeworm, *Echinococcus multilocularis*, whose hosts are canid sp. like the Red fox, raising concerns about human infection (Cunningham et al., 2017).

Wild ruminants, such as deer, are susceptible to infection by generalist helminth species. benzimidazole-resistant *H. contortus* has been found in wild deer in the UK and Hungary. Experimental studies confirm that resistant larvae from wild deer can infect cattle and sheep

(Brown et al., 2022). At the livestock–wildlife interface, gastrointestinal nematode transmission between livestock and wild ungulates in the Himalayas, tick-borne diseases in Botswana, and trypanosomiasis at the wildlife–livestock interface in Kenya (Babayani et al., 2022).

Wild ruminants in the United States—including white-tailed deer, mule deer, elk, caribou, pronghorn, and bighorn sheep—were found to harbor several important gastrointestinal nematode (GIN) parasites commonly associated with domestic cattle. The main parasite genera detected *were Ostertagia, Trichostrongylus*, and *Haemonchus*. The prevalence rate of *Ostertagia* was 90% and *Trichostrongylus* was 69%, and *Haemonchus* in 26%. This highlights that wild ruminants can act as reservoirs for cattle nematodes (Barone et al., 2020).

This comprehensive review paper analyses done on infectious diseases at the wildlife–livestock interface worldwide, says 29% of the diseases studied at these interfaces are parasitic. Notable parasitic diseases include theileriosis, trypanosomiasis, and various gastrointestinal strongyle infections (Wiethoelter, Beltrán-Alcrudo, Kock, & Mor, 2015).

1.5 OBJECTIVES

The objective of this study was to investigate the spatial and temporal patterns of parasite burden (EPG) in Nilgai about their interactions with cattle. Specifically, the study aimed to:

Spatial Objectives

- To quantify and map the spatial distribution of nilgai and cattle, and assess the degree of spatial overlap between the two species.
- To evaluate the influence of spatial proximity to cattle (including infected individuals) on parasite burden (EPG) and prevalence in nilgai.
- To identify and assess environmental risk factors associated with spatial variation in parasite transmission.

Temporal Objectives

- To examine whether temporal activity overlap with cattle is associated with variation in parasite load (EPG) in nilgai

1.6 RESEARCH

Spatial Objectives

- To what extent do nilgai (*Boselaphus tragocamelus*) and cattle exhibit spatial overlap in habitat use within the study area?
- Does proximity to cattle, correlate with increased gastrointestinal parasite burden (measured as eggs per gram, EPG) or prevalence in nilgai populations?

- Which environmental variables (e.g., proximity to water sources, distance from villages) are significantly associated with spatial variation in gastrointestinal parasite transmission risk among nilgai?

Temporal Objectives

- Does temporal overlap in activity patterns between nilgai and cattle influence the intensity or prevalence of gastrointestinal parasite infections (EPG) in nilgai?

CHAPTER 2: STUDY AREA

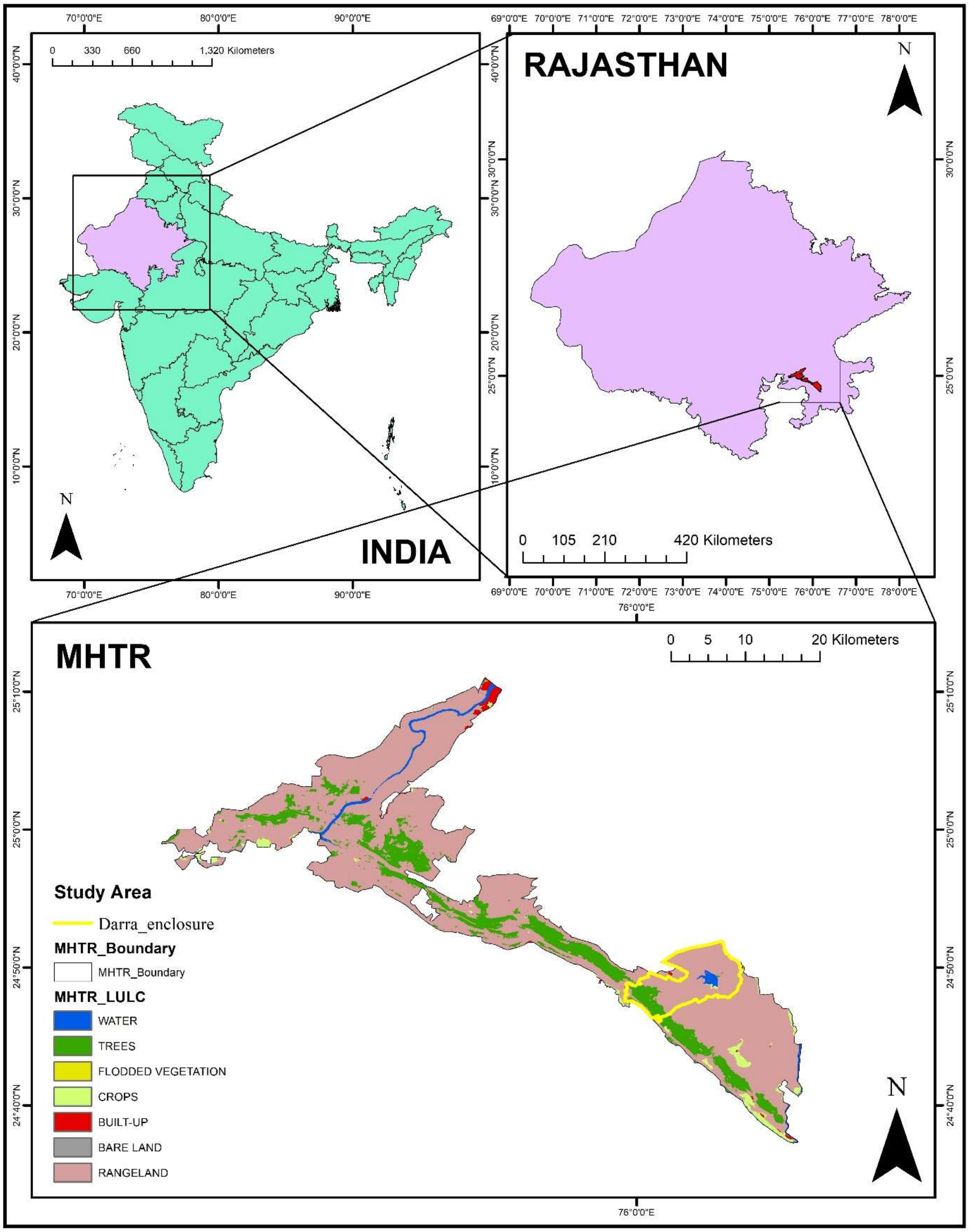

Figure 1: Study area- Mukundara Tiger Reserve Map

DARRA ENCLOSURE

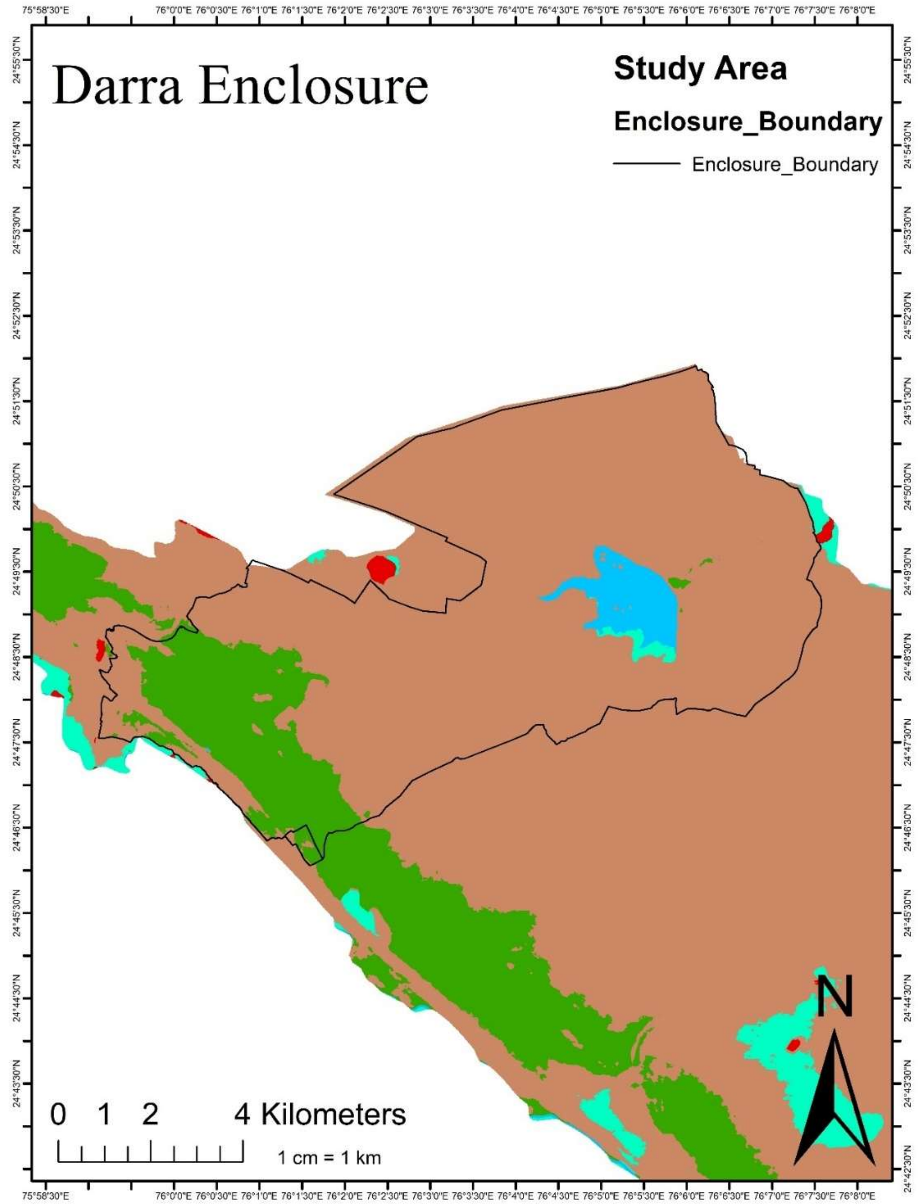

Figure 2: *Darra Enclosure Map*

This study was conducted in the Darrah enclosure (82 km²) within the Mukundara Hills Tiger Reserve (MHTR), Kota district, Rajasthan, India (central coordinates: 24°52′05″N, 75°51′22″E). The Darrah enclosure is a specially managed, predator-proof area established for rewilding and the recovery of wild prey populations to support future large carnivore conservation. The enclosure features flat savanna grassland in the north and hilly, rocky terrain elsewhere, with perennial water sources such as the Sawan Bhado reservoir and Foot Talab.

The climate is semi-arid, with mean annual temperatures between 23.5°C and 29°C, and summer highs reaching up to 48°C. Annual rainfall typically ranges from 1000 to 1300 mm, mostly during the monsoon season (July to September), with mild, dry winters (5–25°C). The topography includes rugged hills, open rocky plateaus, and sparse vegetation. Vegetation is dominated by grasses (*Aristida spp.*, *Dichanthium annulatum*, *Heteropogon contortus*, *Themeda* spp.), with scattered trees such as *Anogeissus pendula*, *Vachellia catechu*, *Flacourtia indica*, and *Senegalia senegal*. Wildlife includes leopard (*Panthera pardus*), grey wolf (*Canis lupus*), striped hyena (*Hyaena hyaena*), Indian fox (*Vulpes bengalensis*), golden jackal (Canis aureus), blackbuck (*Antilope cervicapra*), chinkara (*Gazella bennettii*), nilgai (*Boselaphus tragocamelus*), and sambar (*Rusa unicolor*).

The reserve is surrounded by 14 villages within its boundaries and about 100 villages around its periphery, whose residents rely on forest and grassland resources for cattle grazing, fuelwood, and non-timber forest products, resulting in significant livestock–wildlife interface and grazing pressure

Study duration:

Fieldwork was conducted from January 2025 to March 2025. A total of 96 nilgai fecal samples and 81 cattle fecal samples were collected for parasitological analysis within the Darrah enclosure

CHAPTER 3: STUDY SPECIES

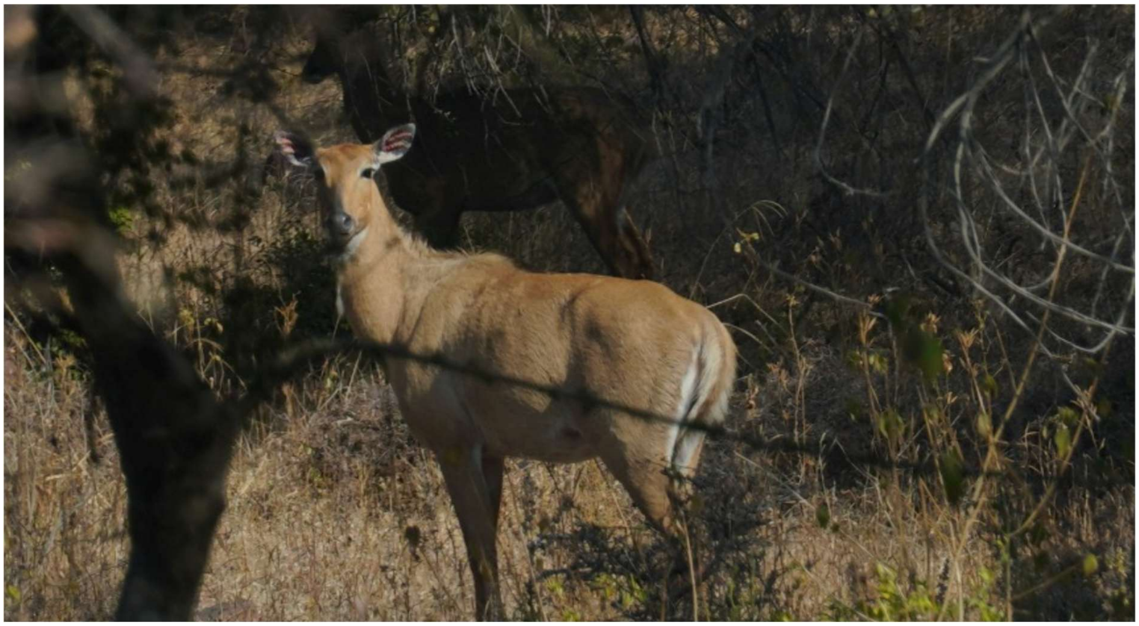

Plate 1*: Boselaphus tragocamelus (Nilgai)*

Taxonomical Classification

Kingdom: Animalia

Phylum: Chordata

Class: Mammalia

Order: Artiodactyla

Family: Bovidae

Genus: *Boselaphus*

Species: *Boselaphus tragocamelus*

Common Name: Nilgai

3.1 DESCRIPTION

The nilgai is the largest antelope in Asia and is indigenous to the Indian subcontinent. Adult nilgai bulls are steel-grey or blue-grey with black legs; calves and females are light brown. The lighter colour of male calves begins to darken by the tenth month, and they develop black legs and brownish-grey shoulders by the 18th month. Males obtain adult coloration by their fourth year (Sheffield et al. 1983). Only males have horns. All ages of both sexes have dark and white markings on their heads, ears, underparts, fetlocks, and tails. Males have a more pronounced tuft of hair at the midpoint on the ventral side of the neck.

3.2: GEOGRAPHICAL DISTRIBUTION

In the past, nilgai were distributed all over the country. The present distribution of nilgai ranges from the Himalayan foothills to the southern extent of Andhra Pradesh. Most of the nilgai population is centered in northwest India. Small population in Nepal and Pakistan, and is considered extinct in Bangladesh. However, the introduced population of nilgai in Texas is doing very well. It was introduced in the 1920s, and today they have expanded to the borderlands between Texas and Mexico *(Joshua, 2019*).

3.3 ECOLOGY

Group size and composition

Nilgai are partly social in their habits. According to Schaller (1967), nilgai groups change constantly. In Northern India, during July to October, all-male groups' herd size can reach 10 individuals or fewer. Herd size of non-breeding females and their calves is usually less than 16, often as single, and occasionally of 16 to 24 individuals (Sankar 1994). Usually, three types of groups are distinguishable: 1 or 2 adult females with young calves, 3 to 6 adult and yearling cows with calves, and male groups varying in number from 2 to 18 (Sankar 2004). Herd size varies by habitat type, as resource availability and distribution influence the herd size. In semi-arid regions, group size varies from 2 to 7 individuals (Khan et al., 1995). Nilgai densities show great variation in terms of season, geography, and resource availability. Nilgai density can go from 6 to 1 individuals per sq. km in Ranthambore National Park (Bagchi et al., 2004). The seasonal variation in density could go from 3.2 individuals per sq. km during the dry season to 5 per sq. km 3.2 (Dinerstein's, 1979).

Food habits

One of the reasons for the wide distribution of nilgai is their wide niche; nilgai can thrive on a wide variety of grasses, herbs, and trees. A study done on the food habits of nilgai in Van Vihar National Park revealed that the nilgai diet consists of plant species, grass forming the major proportion (41.66%), followed by fallen leaves, flowers, and fruits (36.00%), browse (16.66%), and herbs, shrubs, etc. (5.66%). Among the preferred browse species, *Zizyphus mauritiana* had the highest preference rating. It also feeds on leaves and fruits of *Zizyphus mauritiana, Butea monosperma, Acacia nilotica, A. catechu,* and *A. leucophloea*, and leaves of *Anogeissus pendula* (Saheel et al., 2012).

The diet of the nilgai varies from season to season (Saheel et al., 2012). Grass forms an important component of the nilgai's diet (88 percent). They also often raid crops and are considered pests in agricultural fields (Rodgers 1988). Studies in Asia on nilgai food habits show that they are browsers or mixed feeders. In Sariska, nilgai fed on 91 plant species that included 20 trees, 9 shrubs, 12 creepers/stragglers, 29 herbs, and 21 grasses (Sankar, 1994; Johnsingh et al., 2012).

Habitat use and home range

Nilgai are found in open landscapes (Berwick 1974); they avoid dense hilly forests and prefer scrublands with low tree and shrub densities. A study in Sariska showed the home range of a female nilgai to be 3.6 sq km, and its annual home range was 7.3 sq km (Johnsingh et al., 2012). In Texas, Nilgai had a 0.6 to 8.1 sq km home range (Leslie,2008).

In India, nilgai are generally considered diurnal (Brander 1923, Schaller 1967), and the same holds for the Norias (Texas) population, although some degree of nocturnal activity occurred (Sheffield et al. 1983). Dinerstein (1979) reported two activity peaks (at 0700 and 1800 hrs) for nilgai during the hot season in Nepal. In Sariska, a radio-collared female showed two activity peaks: 0700 to 0900 hrs and 1600 to 1800 hrs, during winter (Chakraborty 1991).

CHAPTER 4 METHODOLOGY

4.1 SPATIAL STRATIFICATION (FIELD METHODOLOGY)

The study area was divided into 2×2 km grids to systematically collect fecal samples. Sampling was conducted during the dry season (January to March), when nilgai movements are relatively stable. In semi-arid landscapes, limited resources during the dry season cause herbivores to localize and congregate around stable resource patches like waterholes and residual forage, resulting in more consistent spatial movement patterns. (Courbin et al., 2015; Hunt et al., 2007). Studies on nilgai ecology from both India and Texas support this spatial stratification approach. For example, in Sariska Tiger Reserve, a radio-collared female nilgai had a seasonal home range of 3.6 km² and an annual home range of 7.3 km² (Sankar, 1994). n Texas, Moczygemba et al. (2014) reported no significant difference in home range size between males and females, with maximum home ranges of 16.3 km² for males and 13.8 km² for females, and mean minimum daily movements of GPS-collared females ranging from 1.5 km in January to 2.8 km in May.

This grid-based sampling is also supported by previous studies in wildlife parasitology and ecological research. Which have effectively used 2×2 km grids to collect fecal or scat samples for parasitological, genetic, and behavioural analyses (e.g., Sergeant et al., 2001; Strindberg et al., 2018). Using such a design enhances representativeness, reduces the likelihood of resampling the same individuals, and ensures comparability across habitats, while maintaining a feasible sampling effort in terms of time and resources. Thus making 2×2 km grid sampling ecologically relevant and more of a logistical practical for this study.

4.2 COLLECTION OF FECAL SAMPLES

Fecal samples were collected systematically from January 2025 to March 2025 following a systematic grid-based sampling. Within each grid, random walks are conducted to collect fresh dung and pellet samples. For each fecal sample, date, GPS coordinates, and species were recorded and labelled in the field. Species identification based on fecal samples is done visually by colour, odour, and size. Samples were collected using sterile collection sticks and stored in 10% Formalin solution. Approximately 2 grams of faeces were collected from each individual and stored in 10% formalin and stored at 4 °C until laboratory analysis. his preservation protocol was used to maintain the structural integrity of parasitic eggs, larvae, and cysts, ensuring accurate identification under a microscope.

4.3 METHOD FOR QUANTIFYING SPATIAL OVERLAP VIA GEOGRAPHIC PROXIMITY

Spatial overlap between nilgai and cattle was quantified using a proximity-based approach, leveraging the GPS coordinates (latitude and longitude) of all collected fecal samples. For each nilgai sample, the geographic distance to the nearest cattle sample and to the nearest infected cattle sample (defined as cattle with EPG > 0) was calculated using the Haversine formula.

Samples were grouped based on their proximity to cattle and to infected cattle. Epidemiologically, proximity to infected or carrier individuals plays an important role in influencing parasite transmission dynamics, as spatial overlap increases the likelihood of direct or indirect contact and chances of pathogen spillover. In this study individual with an EPG>0 was considered an infected individual. Thus, proximity to cattle was taken to understand the general exposure risk from livestock. However, proximity to infected cattle was also considered, as the presence of cattle in an area does not necessarily confirm parasite presence.

Thus, focusing on infected cattle helps to better identify zones with a higher risk of parasite transmission. Under this approach, groupings were done under the threshold distances (e.g., within 1000 m, 1000–2000 m, and beyond 2000 m).

The two grouping schemes — a simpler two-group division (overlap vs. non-overlap) and a finer three-group division (high, moderate, low overlap) — provide a hierarchical framework to capture varying degrees of exposure risk. This stratification also incorporates a varying home range of nilgai. The second approach primarily examines the proximity of nilgai to infected cattle. Here, samples were grouped based on whether they were within a set distance from infected cattle. If a sample was within this distance, it was put in the overlap group, which means there is a higher chance of direct exposure. Samples that were outside this distance were kept in the non-overlap group. It is important to note that if a sample falls within the threshold distance of cattle that are not infected, it is still considered part of the non-overlap group. This method allowed for a targeted assessment of exposure risk, directly linking the spatial relationship between nilgai and known infected cattle, thereby strengthening the relevance of the analysis in understanding disease transmission dynamics at the wildlife-livestock interface.

This dual-layered spatial classification enhances the ability to detect fine-scale relationships between livestock proximity and parasitic infection patterns in wild ungulates, supporting both epidemiological inference and behavioural ecology perspectives. Looking at the available sample size, threshold distances for grouping fecal samples were carefully selected to balance ecological relevance and sufficient sample representation within each category. A primary cutoff of 1000 meters was chosen to distinguish areas of direct or frequent spatial overlap between wild ungulates and cattle, representing zones of highest potential parasite transmission risk, and beyond 2000 meters were grouped into an area with minimal or no interaction with livestock, serving as a baseline for comparison. The intermediate zone (moderate overlap), 1000 - 2000 meters, was selected to capture the gradient effects in exposure risk. These thresholds also ensured that each group contained an adequate number of samples to maintain statistical power. Thus, these distance-based groupings provide a practical and biologically relevant framework for investigating parasite transmission dynamics in the study area.

4.3 CAMERA TRAP FOR UNDERSTANDING TEMPORAL ACTIVITY OVERLAP

To assess temporal activity overlap between nilgai and cattle, 30 infrared flash cameras (Reconyx Hyperfire HC500, WI 54636, USA) were deployed across the study area. The area was divided into 2 × 2 km grids, and centroid points for camera placement were generated using ArcMap (version 10.5, ESRI 2016), following established protocols (Kukielka et al., 2013; Salvatori et al., 2022). Since these centroid points are generated without prior knowledge of animal distribution, they are random concerning the animals' locations, which will help to observe the interactions and assess their health conditions in an unbiased manner (Tanwar, Sadhu, & Jhala, 2021). Each camera operated continuously throughout the study period, with detections of nilgai and cattle recorded by date and time.

4.4 LABORATORY ANALYSIS OF FECAL SAMPLES

Qualitative analysis

The fecal samples collected in 10% Formalin solution were subjected to parasite eggs, cysts, and larvae etc, detection using techniques like the Fecal Flotation Technique. The flotation test is sensitive for the detection of helminths and protozoans in feces. It works on the principle of concentrating eggs based on their specific gravity. Using a flotation salt/sugar solution with a specific gravity higher than the parasite eggs or oocysts helps them to float on the surface of the solution. Saturated salt solution (400 gm of salt per 1000 ml of distilled water), fecal sample (2 gm), mortar and pestle, tea-strainer. Using gloved hands, the weighed out portion of the fecal sample was put in approximately 5 ml of NaCl solution and mixed well with a mortar and pestle. This well-mixed slurry was then sieved through a tea-strainer, and the fibrous contents of the fecal sample were discarded. The filtrate was transferred to a 15 ml centrifuge tube and filled to the brim to create a positive meniscus. A coverslip was then placed on this, and the set-up was left to stand for 15-20 mins. The coverslip was gently removed from the top of the centrifuge in such a way that the drop of the fecal solution was hanging on the coverslip. This was then placed on a glass slide and observed under a microscope at 10x and 40x magnification. Every slide was scanned until all the fields of the coverslip were covered, which took up to 20-30 minutes per sample. Parasite eggs were photographed for identification. The sedimentation technique is useful to concentrate trematode eggs, which are heavier than nematode eggs. The sedimentation was carried out with the same sample as the one processed for flotation. After flotation is completed, the heavier contents of the fecal sample settle down at the bottom of the test tube. The flotation solution is decanted carefully and refilled with salt solution and allowed to settle again. This is done until a clear supernatant is obtained, and this clear supernatant is carefully discarded. The remaining sediment at the bottom of the test tube is then smeared on a glass slide and covered with a coverslip to examine for any trematode eggs. The presence of parasite larvae and eggs was recorded, and photographs were taken for every unique egg or individual found within a slide. Size, shape, and colour of ova and helminth eggs were recorded in detail and used for further identification of the parasitic taxon. Identification keys were used from published literature for the identification (Soulsby, 1968)

Identification of parasites

The identification of parasites is done by observing the morphological differences in the parasitic eggs described by Soulsby (1968).

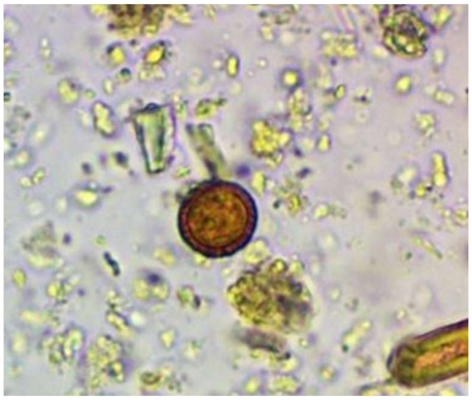

Plate 2*:* Toxocara spp.

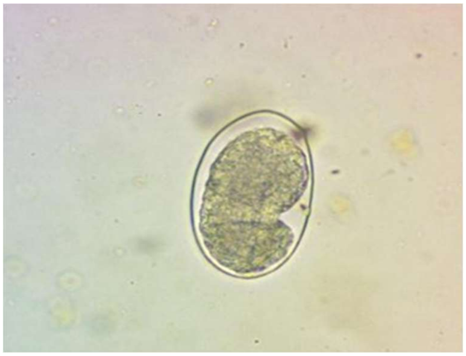

Plate 3*: Strongyloides spp.*

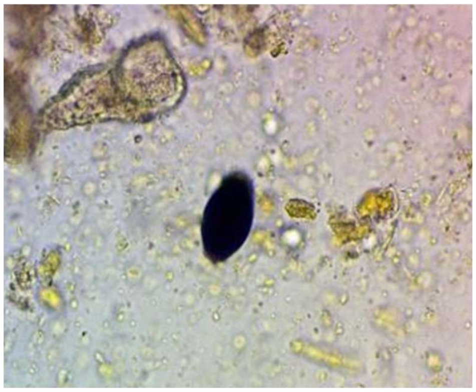

Plate 4: ***Trichuris spp***

4.5 QUANTITATIVE ANALYSIS

To determine the number of eggs present per gram of faeces (eggs per gram/ EPG), the McMaster counting technique (Gordon and Whitlock, 1939) was 2 grams of the fecal sample was thoroughly mixed with 14 ml of the flotation solution and strained. This strained solution was then taken with a dropper and loaded onto the 2 chambers of a McMaster slide. This slide was allowed to rest for 5 minutes for the eggs to float near the surface of the slide for easier detection, and was then observed under a 10x magnification lens.

5. ANALYTICAL METHODS

5.1 PARASITE DATA CALCULATION

The prevalence of parasites in a population is a measure of how many individuals in a population are infected by a particular parasite. This measure just gives an idea about parasite prevalence at the population level.

Prevalence = $(p/n)*100$

where p = number of positive samples, n = total number of samples.

To compare the prevalence rate between the group numbers, a statistical test was done with the help of the software R (version 3.4). This result yielded the proportion of infection in a population, and tested whether these proportions were significantly different in the two areas.

Eggs per gram (EPG) calculation

EPG counts are indicative of the intensity of the infection in an individual. EPG counts were only calculated for the samples that tested positive in the qualitative analysis.

EPG = $(x + y) \times 50$

Where x = number of eggs counted in chamber 1

y = number of eggs counted in chamber 2

5.2 COMPARISONS OF PARASITIC LOAD AMONG SPATIAL OVERLAP GROUPS

To evaluate whether spatial overlap with cattle influenced parasite burden in nilgai, we compared parasite load (measured as eggs per gram, EPG) among groups defined by their proximity to cattle. First, we assessed the distribution of EPG data within each group using the Shapiro-Wilk test to determine whether the data met the assumptions of normality required for parametric tests. As the EPG data were found to be non-normally distributed, we proceeded with non-parametric statistical methods. For comparisons involving two groups (e.g., high vs. low overlap), we used the Wilcoxon Rank-Sum Test (Mann–Whitney U), which is appropriate for comparing medians between independent groups when normality cannot be assumed. For analyses involving more than two groups (high, medium, and low overlap), we employed the Kruskal–Wallis test, the non-parametric equivalent of one-way ANOVA, which is suitable for detecting differences in medians across multiple groups with non-normal distributions. When the Kruskal–Wallis test indicated significant differences, we conducted post-hoc pairwise comparisons using Dunn's test with Bonferroni correction to control for multiple comparisons. All statistical analyses were performed in R (version 3.4) using the stats package for the Wilcoxon and Kruskal–Wallis tests, and the dunn.test package for post-hoc analysis. This stepwise analytical approach was chosen to ensure robust and reliable detection of differences in parasite burden associated with varying degrees of spatial overlap, in accordance with established practices in ecological parasitology.

5.4 MAPPING HOTSPOT AND SPATIAL AUTOCORRELATION ANALYSIS

5.4.1 Hotspots Mapping

GIS software (e.g., QGIS or ArcGIS) to visualize spatial clustering of high parasite loads.

It is important to visualize the spatial distribution of infection intensity to identify potential clusters or hotspots. Hotspot mapping was done to visualize the spatial distribution of infection intensity and to identify potential hotspots. Hotspot mapping involves plotting the observed values (such as EPG counts) on a map to visually assess whether areas with high or low values tend to occur together. This initial step helps in recognizing spatial patterns or localized clusters that may be linked to ecological or environmental factors, such as shared water sources, grazing areas, or movement corridors. The hotspot map will provide an initial visualization of infection intensity, offering insights into potential spatial clusters and areas of elevated parasite burden..

Such visual inspection is useful for guiding further statistical analysis, as it highlights locations that may require targeted management or further investigation.

5.4.2 Spatial Autocorrelation

Spatial autocorrelation tests like Moran’s I are used to check if parasitic intensity levels are spread out randomly or grouped in certain areas. This test is often used in epidemiology to see if infection patterns are clustered, which might happen if animals are getting infected due to local reasons like sharing water sources, grazing areas, or movement patterns. For both Global and Local Moran’s I analyses in my study, each spatial unit was defined as the GPS location of an individual nilgai from which an EPG value was obtained. This allowed me to look for spatial clustering at the scale of individual animals, which makes sense ecologically given how nilgai move and form herds.

Moran’s I assumes that the spatial process is stationary across the study area. However, my study area is quite heterogeneous, which can violate this assumption and limit how much we can interpret from the global Moran’s I results. To address this, I also applied Local Moran’s I (LISA), which lets us detect spatial clusters and outliers at a finer scale, giving a more accurate picture of spatial patterns in habitats that aren’t uniform.

For Global Moran’s I, I chose $k = 4$ (k-nearest neighbour) to define spatial relationships. This number is enough to catch local patterns without making the group too large, which could hide smaller clusters. The idea is that each nilgai’s EPG value is likely to be most similar to those of its four nearest neighbours. From an animal ecology perspective, $k = 4$ fits well with the typical herd size of nilgai, which can range from 2 to 7 individuals in semi-arid regions (Khan et al., 1995), and with their average density in places like Ranthambore National Park (Bagchi et al., 2004). A small k value also helps to focus on local dynamics—choosing a k that’s too big would mix up the data over a larger area and could make us miss small but important clusters. In my analysis, clustering remained significant even when I tried different k values, but $k = 4$ seemed to be a good minimum group size to capture local similarity without over-smoothing.

All spatial autocorrelation analyses, including both Global and Local Moran’s I, were performed in R using the spdep package, which is widely used for spatial statistics and provides robust functions for Moran’s I calculations

5.5 ENVIRONMENTAL RISK FACTOR ANALYSIS

Spearman rank correlation test was done to assess the association of risk factors like distance from the village, water point, with parasitic load and prevalence in nilgai. The Spearman test was used. Since data is non-normal, this non-parametric test assesses the strength and direction of association between two variables that may not follow a normal distribution or have a linear relationship.

The Spearman correlation coefficient (rho, ρ) ranges from -1 to 1, where values close to 1 indicate a strong positive association (as one variable increases, so does the other), values close to -1 indicate a strong negative association (as one increases, the other decreases), and values around 0 indicate no association. The corresponding p-value indicates whether the observed association is statistically significant.

5.6 TEMPORAL ACTIVITY AND PARASITISM ANALYSIS

5.6.1 Overall Activity Overlap Across The Landscape

Camera trap data were used to construct diel activity curves for both nilgai and cattle. To quantify the degree of temporal overlap in their activity patterns, the Coefficient of Overlap (Δ) was calculated. This metric provides an estimate of how much the activity periods of the two species coincide over a 24-hour cycle, with values ranging from 0 (no overlap) to 1 (complete overlap). All analyses for activity pattern estimation and overlap calculation were performed in R using the overlap package, which is specifically designed for analyzing and visualizing temporal activity patterns from camera trap data.

5.6.2 Parasitic Prevalence And Activity Relationship Across The Gradient Of Overlap In The Landscape

To assess whether temporal activity overlap between nilgai and cattle influenced parasite burden, parasitological data were integrated with camera trap records at spatial grid locations. Each nilgai fecal egg count (EPG) sample was assigned to its nearest camera trap grid using spatial nearest-neighbor analysis. Within each grid, temporal overlap between nilgai and cattle was quantified as the proportion of shared active hours per day, calculated using the *lubridate* package in R. The temporal overlap index (ranging from 0 to 1) was derived for each spatial grid, with values in this study ranging from 0 to 0.4. Grids with nilgai activity but no cattle presence were categorized as **NA** (not applicable).

The temporal overlap index was merged with the nilgai EPG dataset, enabling analysis of each sample in the context of local cattle presence. Samples were categorized into overlap groups (e.g., low, medium, high), and non-parametric tests (Wilcoxon rank-sum or Kruskal-Wallis) were used to compare EPG across groups. Spearman's rank correlation was applied to assess the relationship between continuous overlap values and EPG.

Stricter grouping criteria were applied to grids based on the degree of temporal overlap, informed by epidemiological principles recognizing both direct and indirect parasite transmission routes. Even in the absence of direct temporal overlap, sequential use of shared spaces can facilitate transmission via environmental contamination (Sun et al., 2016).

Grouping Criteria:

- **No Cattle (NA):** Grids where only nilgai were detected and cattle were absent. In these grids, the risk of cattle-to-nilgai parasite transmission is negligible, as there is no environmental contamination from cattle.

- **No Temporal Overlap (0):** Grids where both cattle and nilgai were detected, but their activity periods did not overlap at all (overlap coefficient = 0). Both species use the same space, but not at the same time. While the risk of direct transmission is absent, there remains a possibility of indirect transmission if parasites persist in the environment.
- **Temporal Overlap (>0):** Grids where both species were detected at the same time and at least some temporal overlap in their activity occurred (overlap coefficient > 0). These situations represent the highest likelihood of direct or near-direct contact and, therefore, the greatest risk of parasite transmission.

This classification reflects the understanding that parasite transmission can occur through both direct encounters and environmental contamination, with the likelihood increasing when both species are present and active simultaneously (VanderWaal & Ezenwa, 2016; Sun et al., 2016)

All analyses were conducted in QGIS and R (version 4.3.1) using packages including sf, geosphere, spdep, ggplot2, and MASS. Non-parametric tests were applied where the data were non-normally distributed. Statistical significance was set at $\alpha = 0.05$.

6. RESULT

This study aims to investigate the parasitic load and prevalence in nilgai and cattle by examining their spatial and temporal overlap with livestock at the livestock-wildlife interface

6.1 SPATIAL EXTENT OF HABITAT USE BY NILGAI AND CATTLE

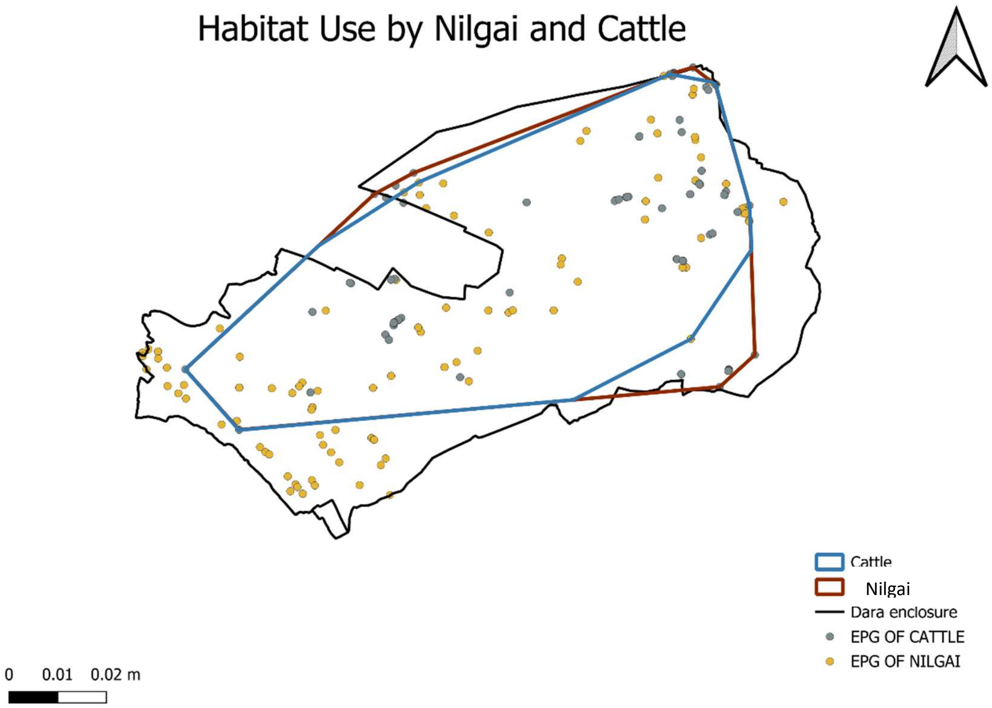

Figure 3: Habitat Use Map

The spatial extent of habitat use for nilgai and cattle was estimated using the Minimum Convex Polygon (MCP) method, based on the locations of indirect signs (dung) recorded during field surveys. The MCP area for nilgai was **6,838.77 hectares (68.39 km²)**, while the MCP area for cattle was **6,129.73 hectares (61.30 km²)**. The area both used (overlap) is about **5607.928 hectares** (**56.08 km²)**. The MCP approach provides a standardized metric for comparing the overall space used by Nilgai and cattle.

6.2 INFLUENCE OF CATTLE PROXIMITY ON PARASITIC LOAD OF NILGAI (GENERAL PROXOMITY).

6.2.1: Descriptive Statistics Statistics For Nilgai Parasitic Load By Proximity To Cattle

a. Two Group Comparison

Proximity Group	**n**	**Mean EPG**	**Median**	**SD**	**Min EPG**	**Max EPG**	**IQR (EPG)**
High	75	161.0	150	189.0	0	1500	100
Low	21	64.3	50	79.3	0	300	100

Table 1: Descriptive statistics for nilgai parasitic load by to cattle (Two-Group Comparison*)*

Interpretation

Descriptive statistics were calculated for EPG in Nilgai, grouped by proximity to cattle.
For the two-group comparison, Nilgai within 1,000 meters of any cattle ("High") had a higher mean and median EPG compared to those farther away ("Low").

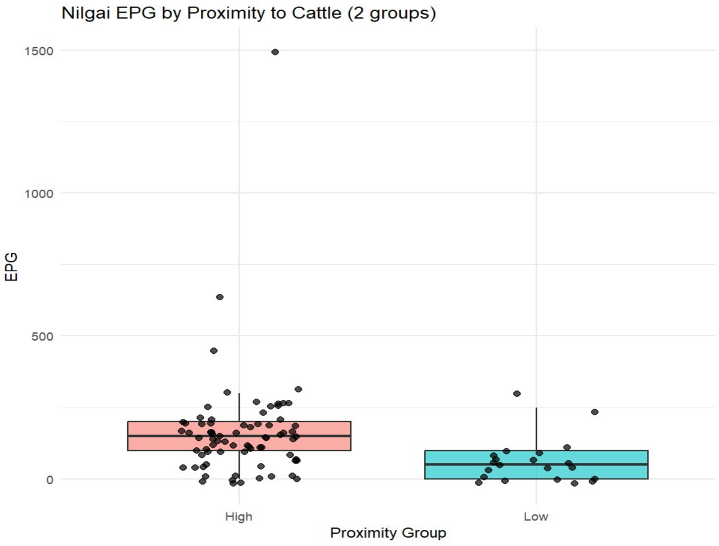

Figure 4: Boxplot showing the distribution of parasitic load in Nilgai by proximity to Cattle

b. Three Group Comparison

Proximity Group	n	Mean EPG	Median	SD	Min EPG	Max EPG	IQR EPG
High (≤1,000 m)	75	161.0	150	189.0	0	1500	100
Medium (1,000–2,000 m)	17	79.4	50	81.1	0	300	50
Low (>2,000 m)	4	0	0	0	0	0	0

Table *2*: Descriptive statistics for nilgai parasitic load by proximity to cattle for three-group

Interpretation

The High proximity group (≤1,000 m) had the highest mean (161.0) and median (150) EPG values, followed by the Medium group (mean = 79.4, median = 50). The Low proximity group (>2,000 m) had zero EPG values, but the sample size was very small (n = 4)

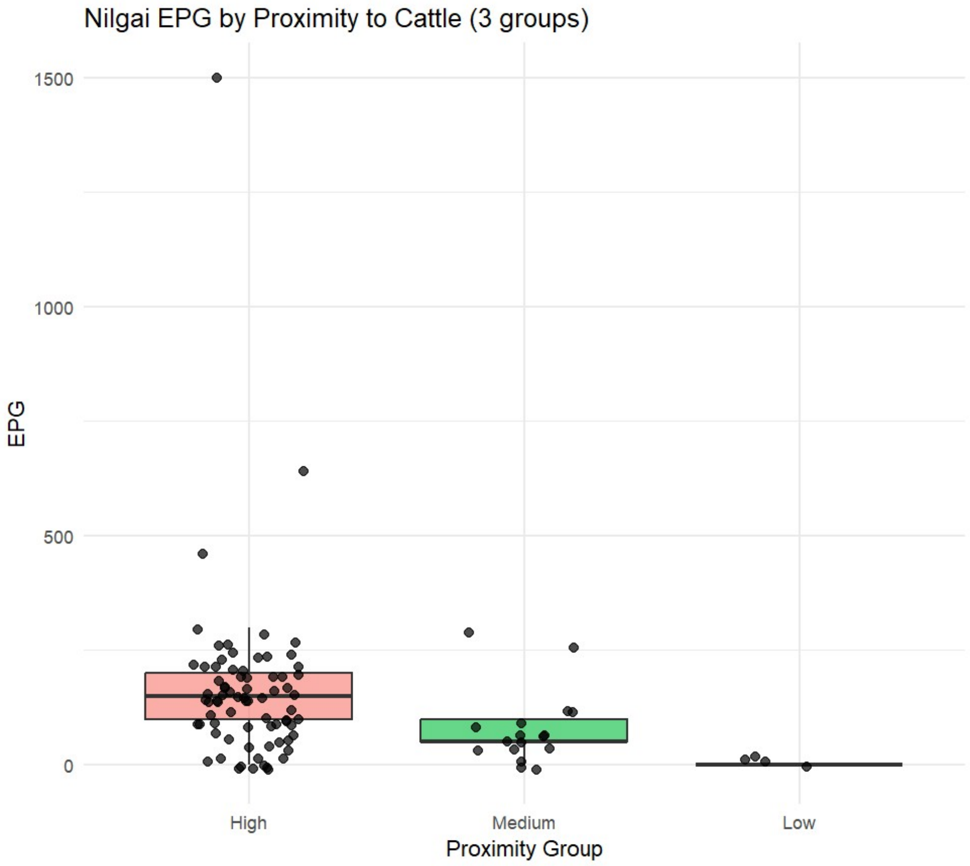

Figure 5:Boxplot showing the distribution of parasitic load in Nilgai by proximity to cattle

6.2.2 Distribution Normality for Nilgai Parasitic Load by proximity to Cattle.

The Shapiro-Wilk test assessed whether EPG values within each proximity group were normally distributed, which is important for selecting appropriate statistical tests.

a. Two groups

Group	W statistic	p-value	Normality
High	0.545	6.822e-14	Not normal
Low	0.730	6.73e-05	Not normal

Table 3: Distribution normality for nilgai parasitic load by proximity to cattle (Two groups)

b. Three groups

Group	W statistic	p-value	Normality	Note
High	0.545	6.822e-14	Not normal	
Medium	0.741	<0.001	Not normal	
Low	-	-	Not testable	All values are identical (EPG = 0)

Table 4: Distribution normality for nilgai parasite load by proximity to cattle (Three groups)

The Shapiro-Wilk test was conducted to assess the normality of EPG (Eggs Per Gram) values in nilgai across proximity groups based on their distance to cattle.

For the two-group comparison (High and Low proximity), the results indicated significant deviations from normality in both groups (High: $W = 0.545$, $p < 0.001$; Low: $W = 0.730$, $p < 0.001$), suggesting that the EPG data were not normally distributed (Table 6.3).

Similarly, for the three-group classification (High, Medium, Low proximity), both the High ($W = 0.545$, $p < 0.001$) and Medium ($W = 0.741$, $p < 0.001$) groups showed significant departures from normality (Table 6.4). The Shapiro-Wilk test could not be performed for the Low group because all EPG values were identical (zero), making the distribution test inapplicable.

Based on these results, non-parametric statistical methods were employed in subsequent analyses.

6.*2.3:* Comparison Of Parasitic Load Of Nilgai In High And Low Proximity To Cattle (Two Groups)

Wilcoxon Rank-Sum Test (Two-Group Comparison) Result

Comparison	Test Statistic (W)	p-value	Significant
High vs Low proximity	1191	0.00030	Yes

Table 5: Comparison of parasitic load values of Nilgai in high and low proximity to Cattle

A Wilcoxon rank-sum test (Mann-Whitney U test) was performed to compare EPG values between nilgai in the high proximity group (≤1,000 m from cattle, n = 75, median = 150) and those in the low group (>1,000 m, n = 21, median = 50). The test indicated a statistically significant difference in EPG values between the two groups (W = 1191, p = 0.00030), with the high proximity group exhibiting higher EPG values.

6.2.4: Comparison Of Parasitic Load Of Nilgai In High And Low Proximity To Cattle (Three Groups)

Kruskal-Wallis Test Results (Three-Group Comparison) Result

Test	(chi-squared)	df	p-value	Significant
Kruskal-Wallis	15.82	2	0.00037	Yes

Table 6: Comparison of parasitic load values of nilgai in high, mediumand low proximity to cattle (Three groups)

A Kruskal-Wallis test was conducted to compare EPG values among nilgai grouped by proximity to cattle (High: ≤1,000 m, n = 75, median = 150; Medium: 1,000–2,000 m, n = 17, median = 50; Low: >2,000 m, n = 4, median = 0). The test revealed a statistically significant difference in EPG values among the three groups ($\chi^2 = 15.82$, df = 2, p = 0.00037), with the high proximity group having the highest EPG values, followed by the medium group, and the low proximity group showing no detectable parasite burden.

6.2.5: Comparison Of Parasitic Load Of Nilgai In High,Medium, And Low Spatial Overlap From Cattle

Pairwise Comparisons Result

Comparison	**Adjusted p-value**	**Significant**
High vs Medium	0.0081	Yes
High vs Low	0.0081	Yes
Medium vs Low	0.0095	Yes

Table 7: Comparison of parasitic load of nilgai in high, medium and low from cattle

Post hoc pairwise comparisons using the Wilcoxon rank-sum test with Benjamini-Hochberg correction revealed statistically significant differences in EPG values between all proximity groups: High (n = 75) vs. Medium (n = 17), p = 0.0081; High vs. Low (n = 4), p = 0.0081; and Medium vs. Low, p = 0.0095. Post hoc pairwise comparisons using the Wilcoxon rank-sum test with Benjamini-Hochberg correction revealed statistically significant differences in EPG values between all proximity groups: High (n = 75) vs. Medium (n = 17), p = 0.0081; High vs. Low (n = 4), p = 0.0081; and Medium vs. Low, p = 0.0095.

6.3: INFLUENCE OF INFECTED CATTLE PROXIMITY ON PARASITIC LOAD OF NILGAI.

(Assesses how being near parasitized cattle influences parasite load in nilgai)

6.3.1: Descriptive Statistics For Nilgai Parasite Load By Proximity To Infected Cattle (Two-Group Comparison)

Two group

Proximity Group	n	Mean EPG	Median	SD	Min	Max	IQR
High	56	200.0	150	202.0	0	1500	62.5
Low	40	56.2	50	71.8	0	300	100

Table 8: Descriptive statistics for nilgai parasitic load by proximity to infected cattle (Two-Group Comparison)

Interpretation

Descriptive statistics were calculated to summarize EPG values in Nilgai grouped by proximity to infected cattle. The “High” group included individuals within 1,000 meters of at least one infected cow (EPG > 0), while the “Low” group included those farther than 1,000 meters.

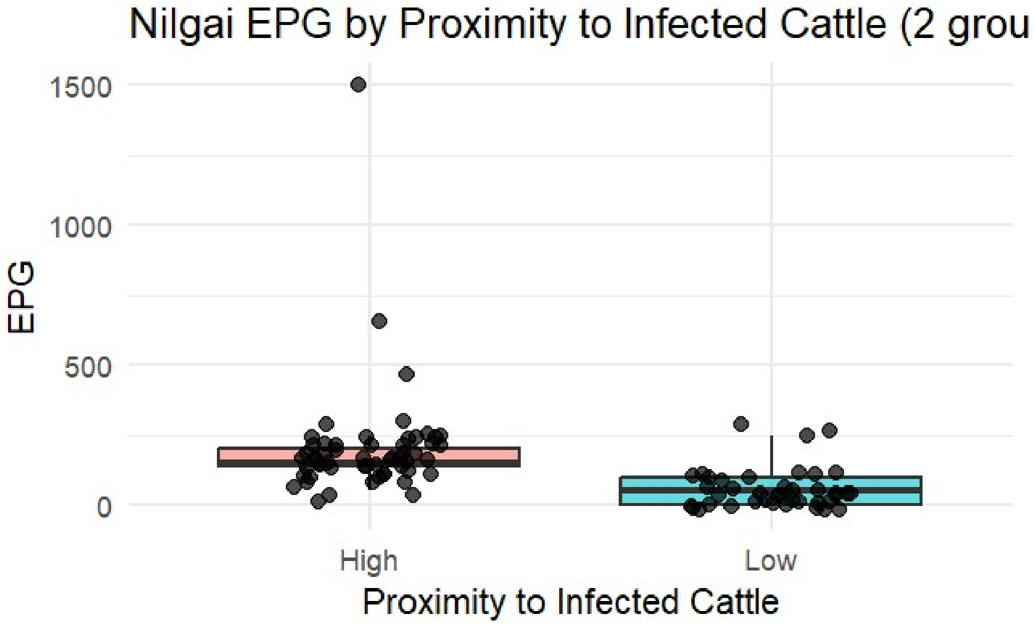

Figure 6:Boxplot showing the distribution of Parasitic Load in Nilgai by proximity to Infected Cattle.

Three Groups

Proximity Group	**n**	**Mean EPG**	**Median EPG**	**SD**	**Min**	**Max**	**IQR**
High	56	200	150	202.0	0	1500	62.5
Medium	22	56.8	50	58.3	0	250	100
Low	18	55.6	25	87.3	0	1030	50

Table 9: Descriptive statistics for nilgai parasitic load by proximity to infected cattle (Three-Group Comparison)

Interpretation

Descriptive statistics were calculated for EPG values among Nilgai grouped by proximity to infected cattle: High (≤1,000 m), Medium (1,000–2,000 m), and Low (>2,000 m).

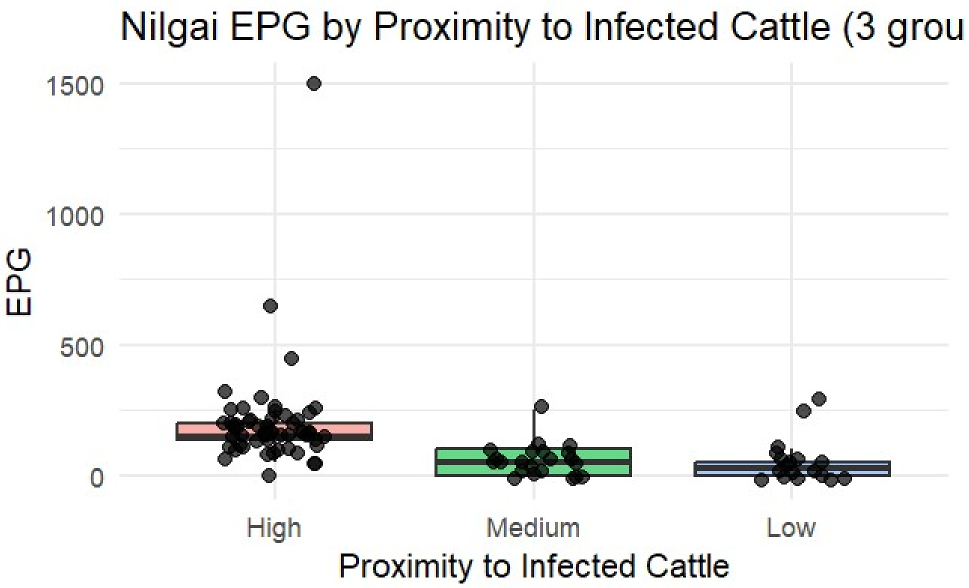

Figure 7:Boxplot showing the distribution of Parasitic Load in Nilgai by proximity to infected cattle

6.3.2: Distribution Normality For Nilgai Parasite Load By Proximity To Infected Cattle (Two Groups).

The Shapiro-Wilk test was used to assess whether EPG values within each proximity-to-infected-cattle group (High and Low) were normally distributed. Evaluating normality is important for determining the appropriate statistical tests for group comparisons.

Two Groups

Proximity Group	Shapiro-Wilk W	p-value	Normality
High	0.727	<0.001	No
Low	0.7258	<0.001	No

Table 10: Distribution normality for nilgai parasitic load by proximity to infected cattle (Two Groups)

Three Groups

Proximity Group	Shapiro-Wilk W	p-value	Normality Assumption
High	0.4744	<0.001	No
Medium	0.7848	<0.001	No
Low	0.6730	<0.001	No

Table 11: Distribution normality for nilgai parasitic load by proximity to infected cattle (Three Groups)

The Shapiro-Wilk test was used to assess the normality of EPG values in nilgai across proximity groups based on distance to infected cattle. In the two-group classification (High and Not_High proximity), both groups showed significant departures from normality (W = 0.727 and 0.726, p < 0.001 for both). Similarly, in the three-group classification (High, Medium, Low proximity), all groups significantly deviated from normal distribution (W = 0.474, 0.785, and 0.673 respectively, p < 0.001 for all). These results indicated that the assumption of normality was violated in all cases, justifying the use of non-parametric statistical methods for subsequent analyses.

6.3.3: Comparison of Parasitic Load of Nilgai in High and Low Proximity to Infected Cattle (Two groups)

Wilcoxon Rank-Sum Test (Two-Group Comparison) Result

Comparison	Test Statistic (W)	p-value	Significant Difference?
High vs Low	1980.5	<0.001	Yes

Table 12: Comparison of parasitic load values of nilgai in high and low proximity to infected cattle (Two Groups)

A Wilcoxon rank-sum test (Mann-Whitney U test) was conducted to compare EPG values between nilgai in the High proximity group (within ≤1,000 meters of at least one infected cow; n = 56, median = 150) and those in the Low proximity group (farther than 1,000 meters from any infected cow; n = 40, median = 50). The test indicated a statistically significant difference in EPG distributions between the two groups ($W = 1982.5$, $p < 0.001$), with nilgai in the High proximity group exhibiting significantly higher EPG values than those in the Low group

6.3.4: *Comparison of Parasitic Load of Nilgai in High and Low Proximity to Cattle* (Three-Group*)*

Kruskal-Wallis Test Results

Test	χ^2 (df)	p-value	Significant
H Vs M Vs L	42.20 (2)	<0.001	Yes

Table 13: Comparison of parasitic load values of nilgai in high, medium and low proximity to infected cattle (Three Groups)

A Kruskal-Wallis test was performed to compare EPG values among nilgai grouped by proximity to infected cattle, using the following distance categories:

- High: within ≤1,000 meters (n = 56, median = 150)
- Medium: 1,000–2,000 meters (n = 22, median = 50)
- Low: >2,000 meters (n = 18, median = 25)

The results indicated a statistically significant difference in EPG distributions across the three groups ($\chi^2 = 42.20$, df = 2, $p < 0.001$), with the High group having the highest EPG values

6.2.2.6: COMPARISON OF PARASITIC LOAD OF NILGAI IN AREAS CLOSE AND FAR FROM INFECTED CATTLE

Pairwise Comparisons Result

Comparison	Adjusted p-value	Significant?
High vs Medium	<0.001	Yes
High vs Low	<0.001	Yes
Medium vs Low	0.37	No

Table 14: Comparison of the parasitic load of nilgai in high, medium, and low from infected cattle

Post hoc pairwise Wilcoxon rank-sum tests with Bonferroni correction revealed significant differences in EPG values between the High group (≤1,000 m) and both the Medium (1,000–2,000 m; $p < 0.001$) and Low (>2,000 m; $p < 0.001$) groups, with the High group exhibiting higher EPG values in both comparisons. No significant difference was found between the Medium and Low groups ($p = 0.37$).

6.4: SPATIAL PROXIMITY AND PARASITE PREVALENCE PATTERNS

6.4.1 Influence Of Cattle Proximity On Parasitic Prevalence In Nilgai

(Prevalence rate comparison based on proximity)

(Two Groups)

Proximity Group	Total Sampled	Infected	Prevalence (%)
High	75	65	86.7
Low	21	14	66.7

Table 15: Prevalence of Infection in nilgai by proximity to cattle (Two Groups)

(Three Groups)

Proximity Group	Total Sampled	Infected	Prevalence (%)
High	75	65	86.7
Medium	17	14	82.4
Low	4	0	0

Table 16: Prevalence of Infection in nilgai by proximity to cattle (Three Groups)

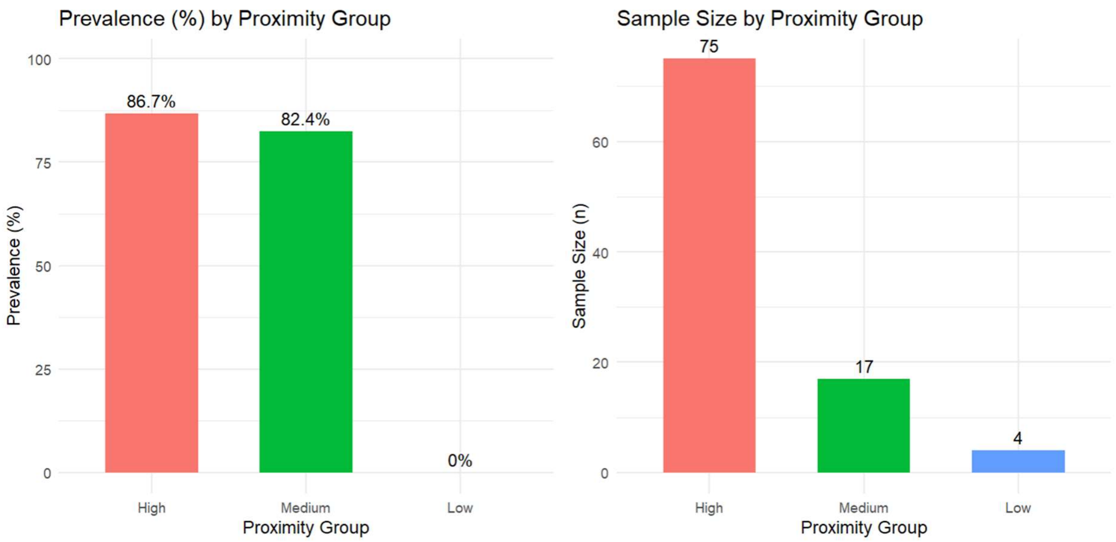

Figure 8: Prevalence (%) and sample size of nilgai parasite load across proximity groups to cattle

The prevalence of infection in Nilgai was compared across proximity groups. In the two-group classification, the prevalence was 86.7% in the high proximity group and 66.7% in the low proximity group. When proximity was categorized into three groups, prevalence was highest in the high proximity group (86.7%), followed by the medium group (82.4%), and was 0% in the low proximity group.) The observed 0% prevalence could be due to the small number of animals sampled rather than a true absence of infection. Bar graphs displaying both prevalence and sample size for each group are provided to aid interpretation

Additional Notes:

The observed 0% prevalence in the low proximity group (three-group comparison) should be interpreted with caution, as the small sample size may not accurately represent the true infection risk in this category. Bar graphs displaying both prevalence and sample size provide important context for interpreting these patterns.

6.4.2 Influence Of Infected Cattle On Parasitic Prevalence In Nilgai

(Prevalence influenced by overlap with infected cattle)

(Two Groups)

Proximity to Infected Cattle	Total Sampled	Infected	Prevalence (%)
High	54	53	98.1
Low	42	26	61.9

Table 17: Prevalence of infection in nilgai by proximity to infected cattle (Two Groups)

(Three Groups)

Proximity to Infected Cattle	Total Sampled	Infected	Prevalence (%)
High	54	53	98.1
Medium	24	17	70.8
Low	18	9	50

Table 18: Prevalence of infection in nilgai by proximity to infected cattle (Three Groups)

The prevalence of infection in Nilgai was highest in the group with high proximity to infected cattle (98.1%), followed by the medium proximity group (70.8%), and lowest in the low proximity group (50.0%)

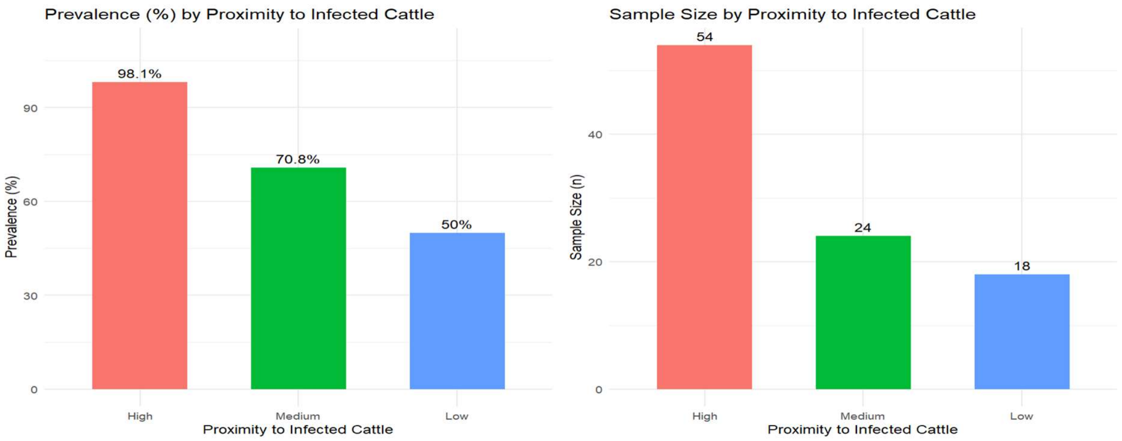

Figure 9: Prevalence (%) and sample size of nilgai parasite load across proximity groups to infected cattle

6.5: INFLUENCE OF ENVIRONMENTAL VARIABLES ON PARASITIC PREVALENCE AND PARASITIC LOAD IN NILGAI

6.5.1. Influence Of Environmental Variables On Parasitic Prevalence

Spearman Correlation Test Result

Variable Pair	Spearman's rho	p-value	Interpretation
Dist cattle_vs Prevalence	-0.354	0.000403	Significant negative correlation
Dist water_vs Prevalence	0.2989	0.003099	Significant positive correlation
Dist village vs Prevalence	-0.1256	0.2229	Not significant

Table 19: Influence of environmental variable on prevalence of nilgai

Spearman correlation analysis showed that Nilgai parasite prevalence was significantly negatively correlated with distance to cattle (rho = -0.354, p < 0.001) and significantly positively correlated with distance to water (rho = 0.299, p = 0.003). No significant correlation was found between distance to village and parasite prevalence (rho = -0.126, p = 0.223).

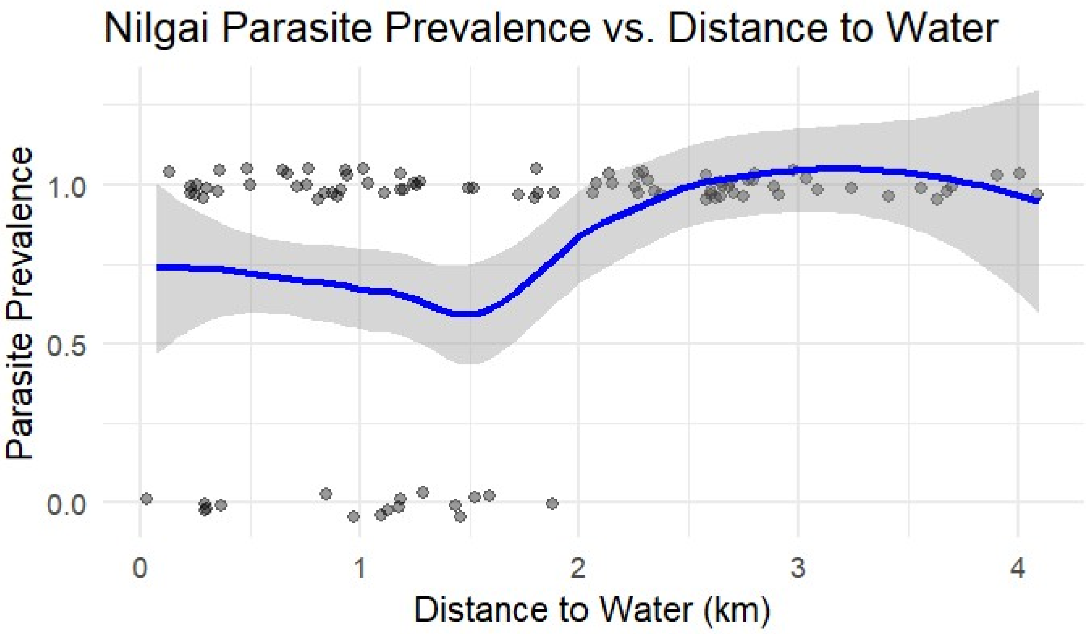

Figure 10: Parasite prevalence in nilgai from water point

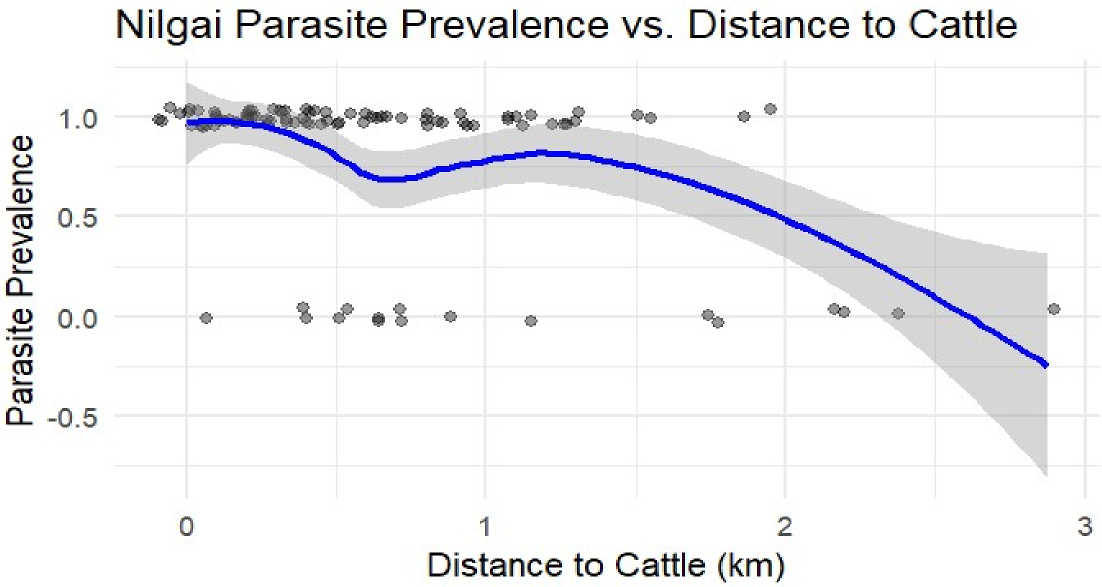

Figure 11 : Parasite prevalence in nilgai from cattle

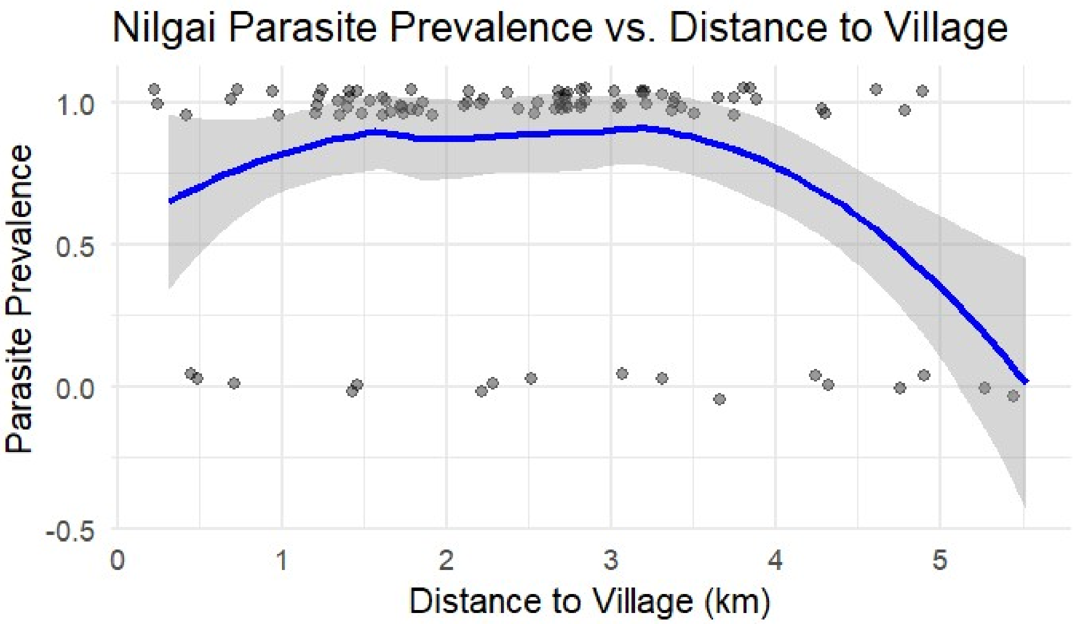

Figure 12: Parasite Prevalence in nilgai from Village.

6.5.2: Influence Of Environmental Variables On Nilgai Parasite Load.

Spearman Correlation Test Result

Variable Pair	Spearman's rho	p-value	Interpretation
Dist cattle vs EPG	-0.4841	5.82E-07	Significant negative correlation
Dist water vs EPG	0.2583	0.01107	Significant positive correlation
Dist village vs EPG	-0.1054	0.3068	Not significant

Table 20: Influence of environmental variables on nilgai parasite load

Spearman correlation analysis revealed a significant negative correlation between distance to cattle and parasite load (EPG) (rho = -0.484, $p < 0.001$) and a significant positive correlation between distance to water and parasite load (rho = 0.258, p = 0.011). No significant correlation was observed between distance to village and parasite load (rho = -0.105, p = 0.307).

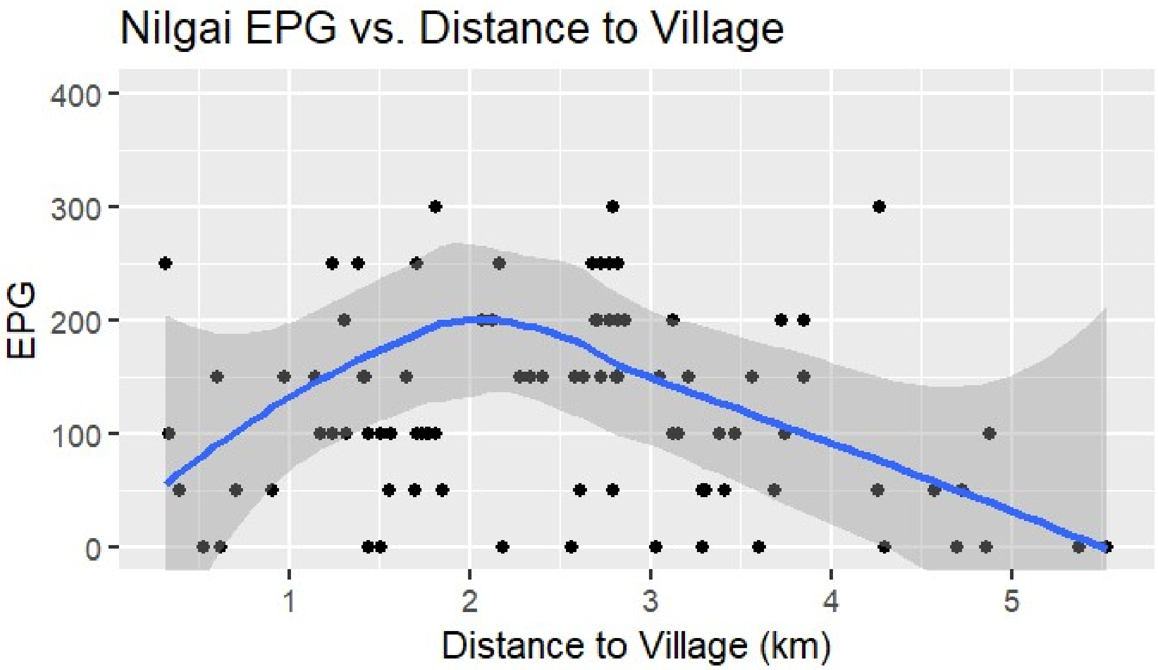

Figure 13: Prevalence of parasite load (EPG) from Village

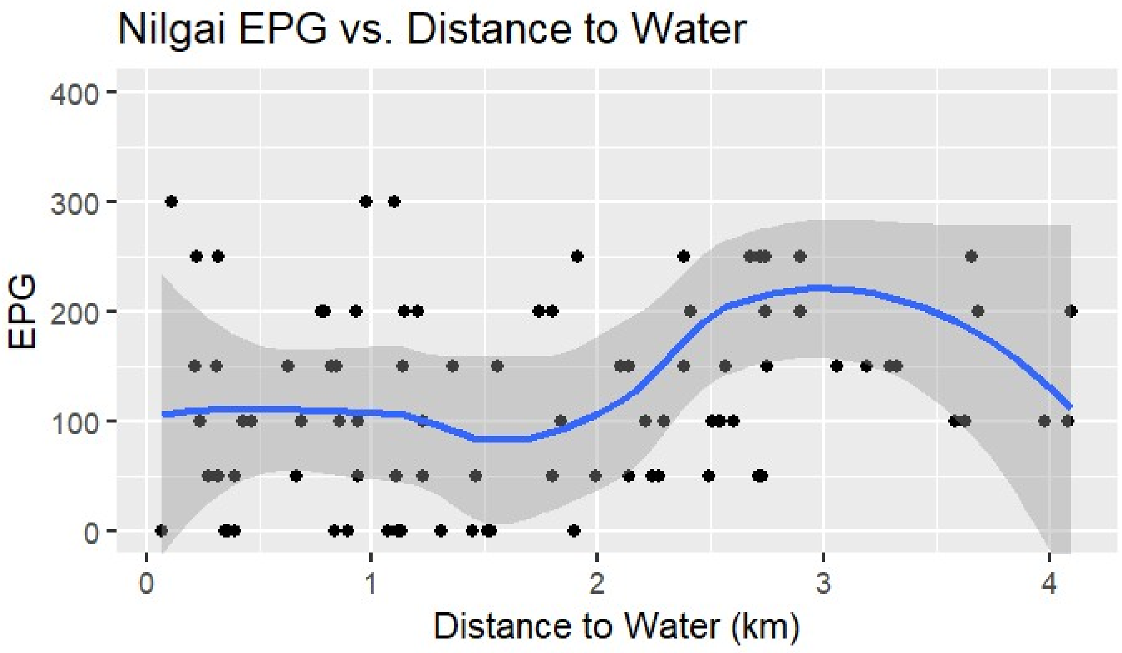

Figure 14: Prevalence of parasite load (EPG) from water

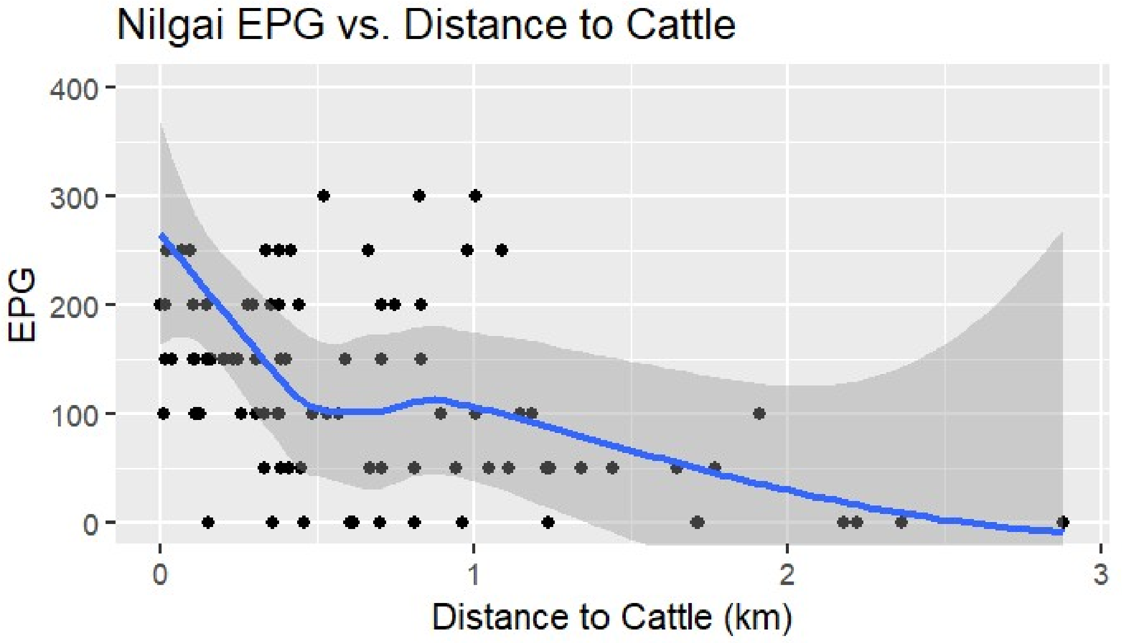

Figure 15: Prevalence of parasite load (EPG) from cattle

6.6: INFLUENCE OF ENVIRONMENTAL VARIABLES ON CATTLE PARASITE PREVALENCE AND PARASITIC LOAD

6.6.1: Influence Of Environmental Variables On Cattle Parasite Prevalence

Spearman Correlation Test Result

Variable Pair	Spearman's rho	p-value	Interpretation
Dist vill_vs Prevalence	-0.206	0.0654	Not correlation
Dist water vs Prevalence	0.0533	0.636	Not significant

Table 6.20: Influence of Environmental Variables on Cattle Parasite Prevalence

Spearman correlation analysis showed that neither variable was significantly correlated with cattle prevalence. Specifically, distance to village showed a weak, negative correlation (Spearman's ρ = -0.206, p = 0.065), while distance to water showed a very weak, positive correlation (Spearman's ρ = 0.053, p = 0.636). In both cases, the p-values exceeded the conventional threshold for statistical significance ($p < 0.05$)

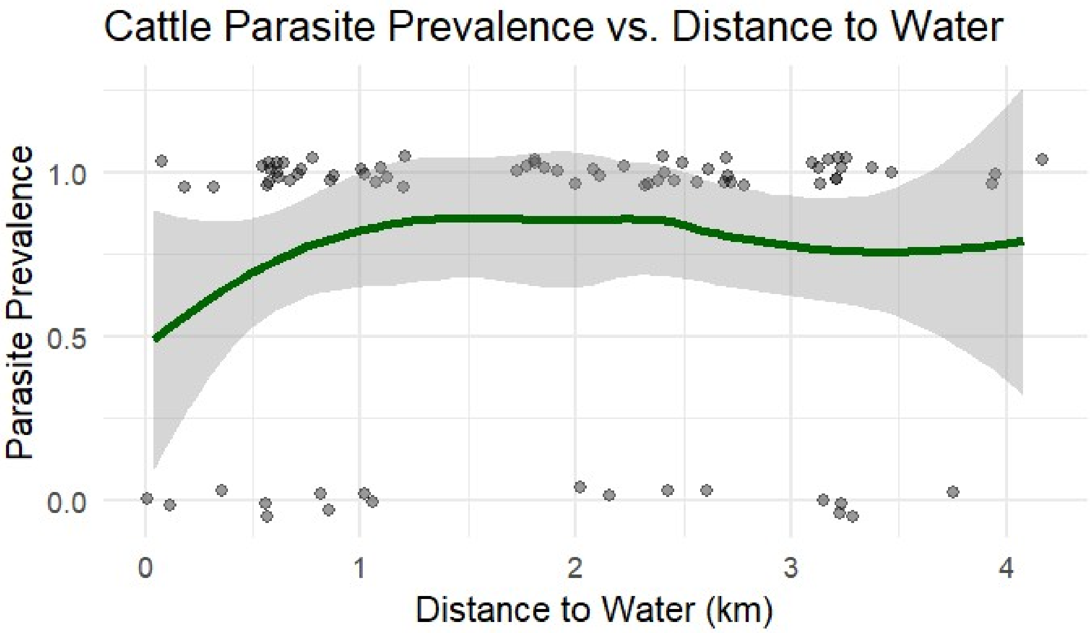

Fig 6.11: Parasite Prevalence from water point

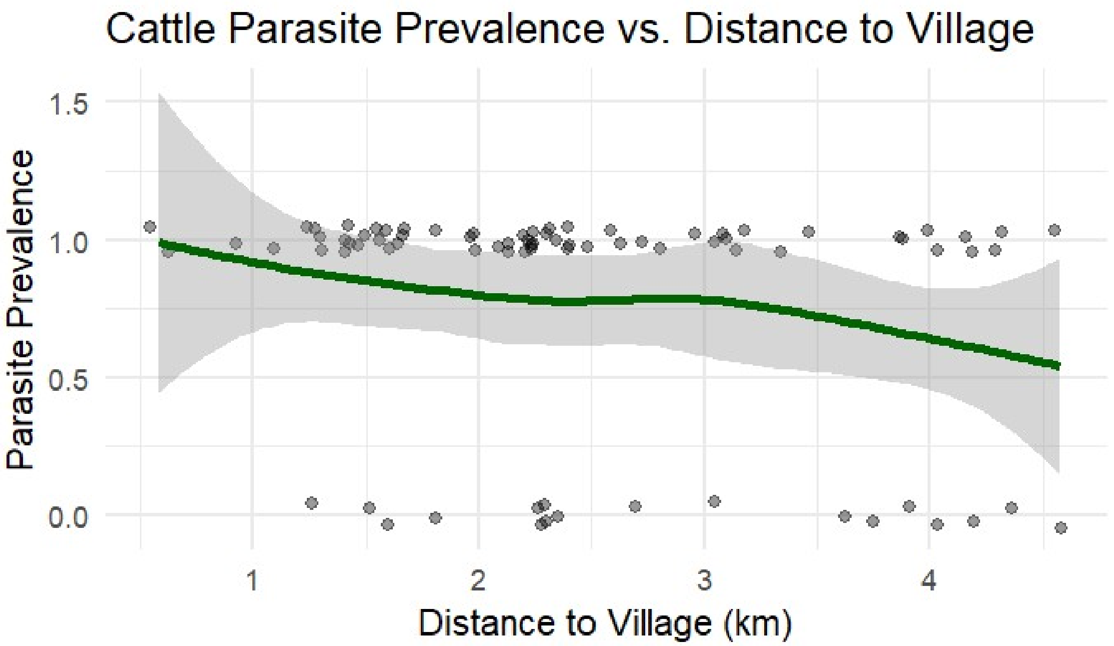

Fig 6.12: Parasite Prevalence from Village

6.6.2: Influence Of Environmental Variables On Cattle Parasite Load

Spearman Correlation Test Result

Variable Pair	Spearman's rho	p-value	Interpretation
Dist water vs EPG	0.063	0.574	Not significant
Dist village vs EPG	-0.139	0.215	Not significant

Table 21: influence of environmental variables on cattle parasite prevalence

Spearman correlation analysis revealed a weak, negative correlation between distance to village and cattle EPG (Spearman's ρ = -0.139, p = 0.215), and a very weak, positive correlation between distance to water and cattle EPG (Spearman's ρ = 0.063, p = 0.574). Neither correlation was statistically significant ($p > 0.05$).

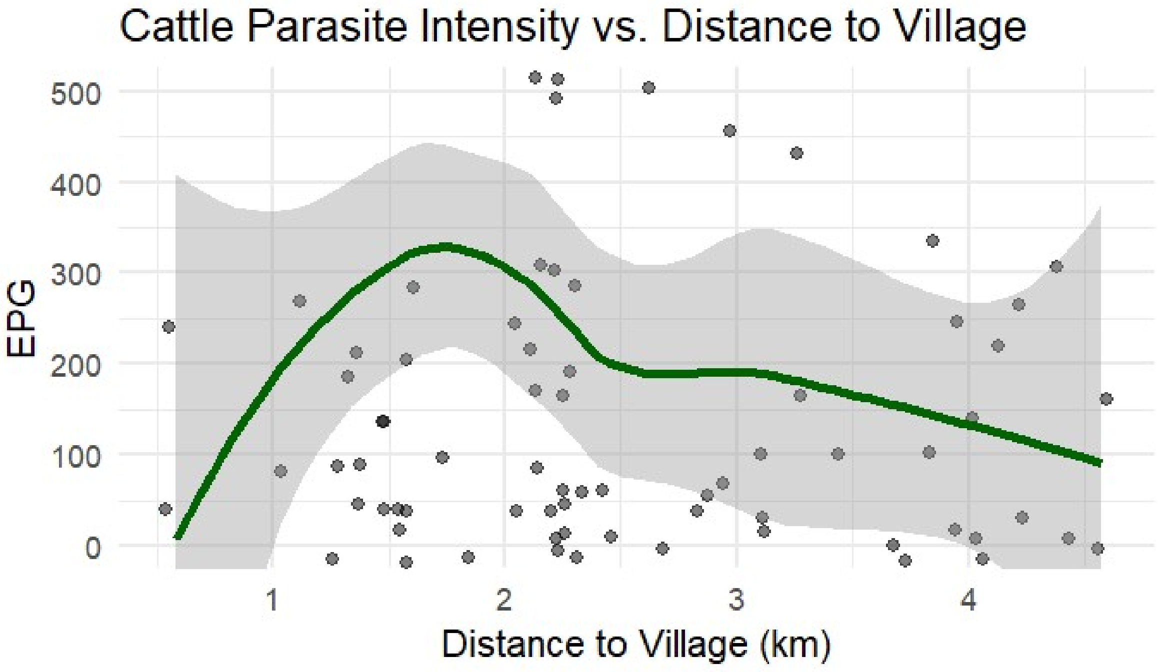

Figure 16: Parasite load (EPG) from village

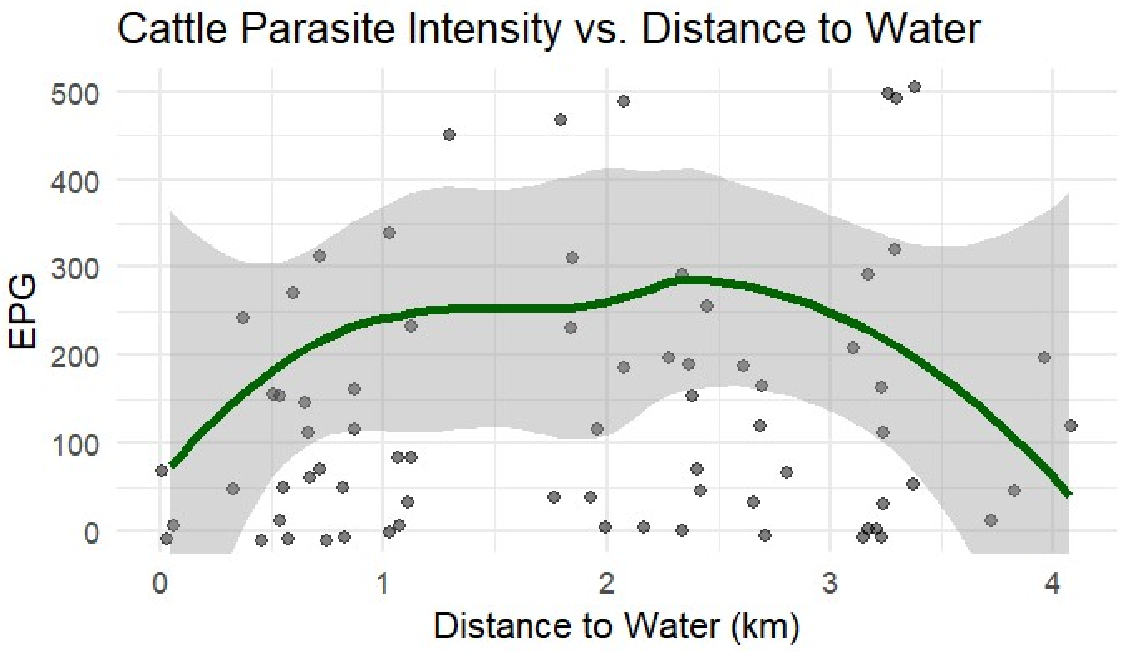

Figure 17: Parasite load (EPG) from water

6.7: SPATIAL PATTERNS OF PARASITIC LOAD

6.7.1: Hotspot Mapping Of Parasite Load In Cattle

(Hotspot analysis for cattle)

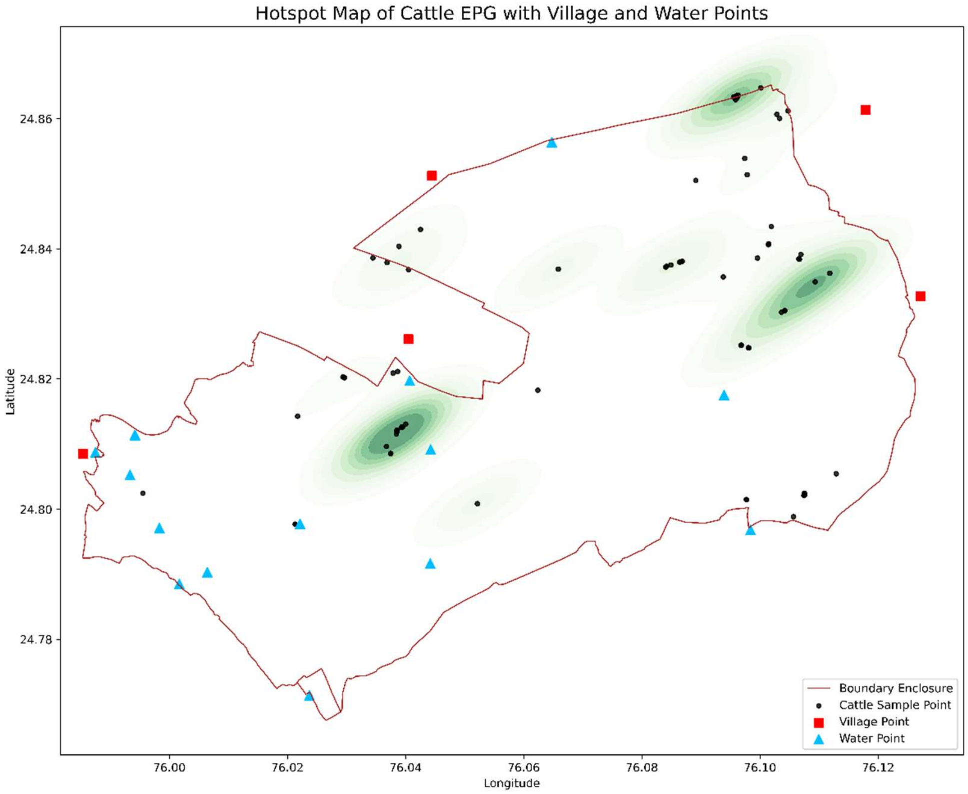

Figure 18: Cattle Hotspot Map

This map illustrates the spatial distribution of gastrointestinal parasite load/Parasitic intensity of cattle within the study enclosure. The grey points represent the locations where cattle fecal samples were collected. The red-to-white gradient indicates the intensity of EPG, with red areas signifying hotspots of high cattle EPG and white areas indicating lower EPG values.

The analysis reveals a non-uniform distribution of cattle parasite load across the enclosure. Noticeable hotspots of high cattle EPG are concentrated close to the boundary enclosure. Conversely, other areas show relatively lower cattle EPG.

6.7.2: Hotspot Mapping Of Parasite Load In Nilgai

(Hotspot analysis for nilgai)

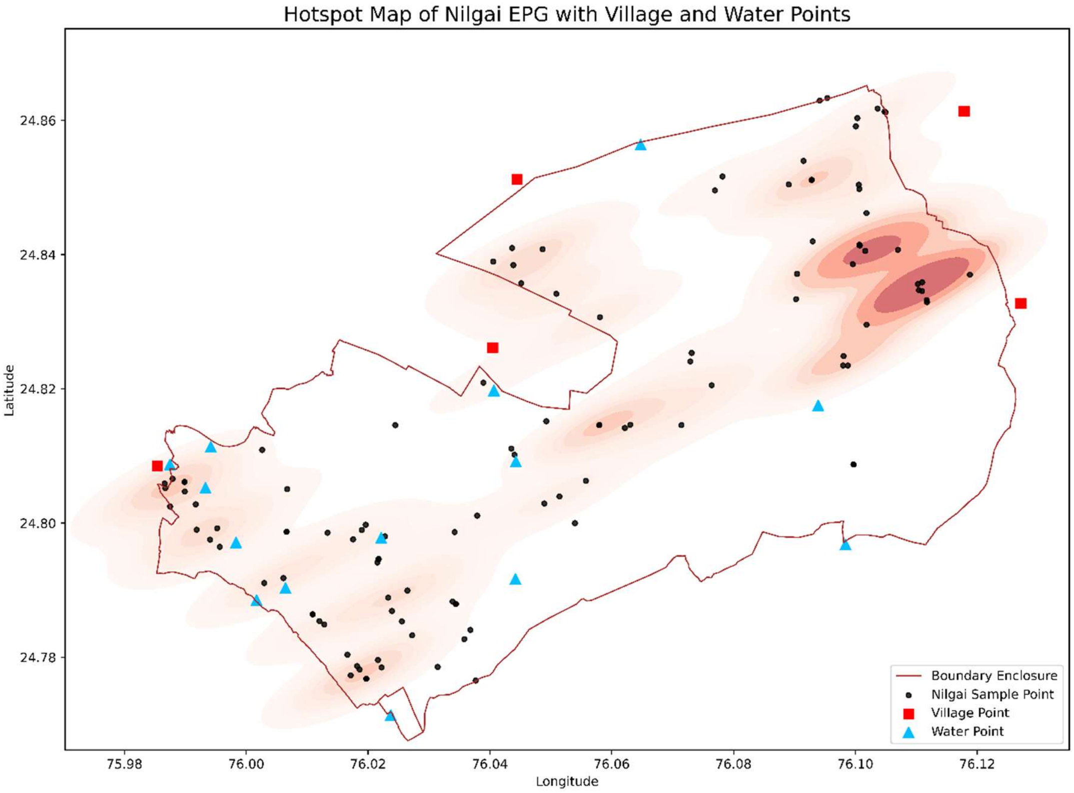

Figure 19: Nilgai Hotspot Map

EPG of Nilgai within the study enclosure. Similar to the cattle EPG map, the grey dots indicate the locations where Nilgai fecal samples were collected. The red-to-white color gradient illustrates the intensity of Nilgai EPG, with red areas representing hotspots of high parasite load and white areas indicating lower levels. Reveals a heterogeneous distribution of Nilgai parasite burden across the enclosure.

6.8: GLOBAL SPATIAL AUTOCORRELATION (MORAN'S I) OF NILGAI PARASITE LOAD, USING NEIGHBORHOOD APPROACHES

Global Spatial Autocorrelation (Moran's I)

Statistic	**Value**	**Test Details**	**Interpretation**
Morana's I	0.124	Monte Carlo permutation (9,999 simulations), k = 4	Positive spatial autocorrelation (clustering)
p-value	0.016		Statistically significant ($p < 0.05$)
Sample size (n)	96	EPG values from nilgai samples	

Table 22: Global spatial autocorrelation (moran's i) of nilgai parasite load

6.8.1: Global Moran's I Test

EPG data is not normally distributed; thus, significance for Moran's I was assessed using a Monte Carlo permutation test (9,999 simulations). This non-parametric approach does not require the assumption of normality and provides a robust p-value for spatial autocorrelation to assess spatial clustering of parasite intensity. A Monte Carlo permutation test for global Moran's I was conducted on EPG values from 96 nilgai samples, using a 4-nearest neighbour spatial weights matrix. The observed Moran's I statistic was **0.124** with a permutation-based p-value of **0.016** (9,999 simulations). The test indicated a significant positive spatial autocorrelation in Nilgai EPG values (Moran's I = 0.124, expected = -0.011, variance = 0.0025, standard deviation = 2.66, p = 0.0039). Stating statistically significant positive spatial autocorrelation in EPG values, Parasite loads were more similar among spatially proximate individuals than expected by chance

6.8.2: Identification of Hotspots, Coldspots, for Nilgai Parasite Load

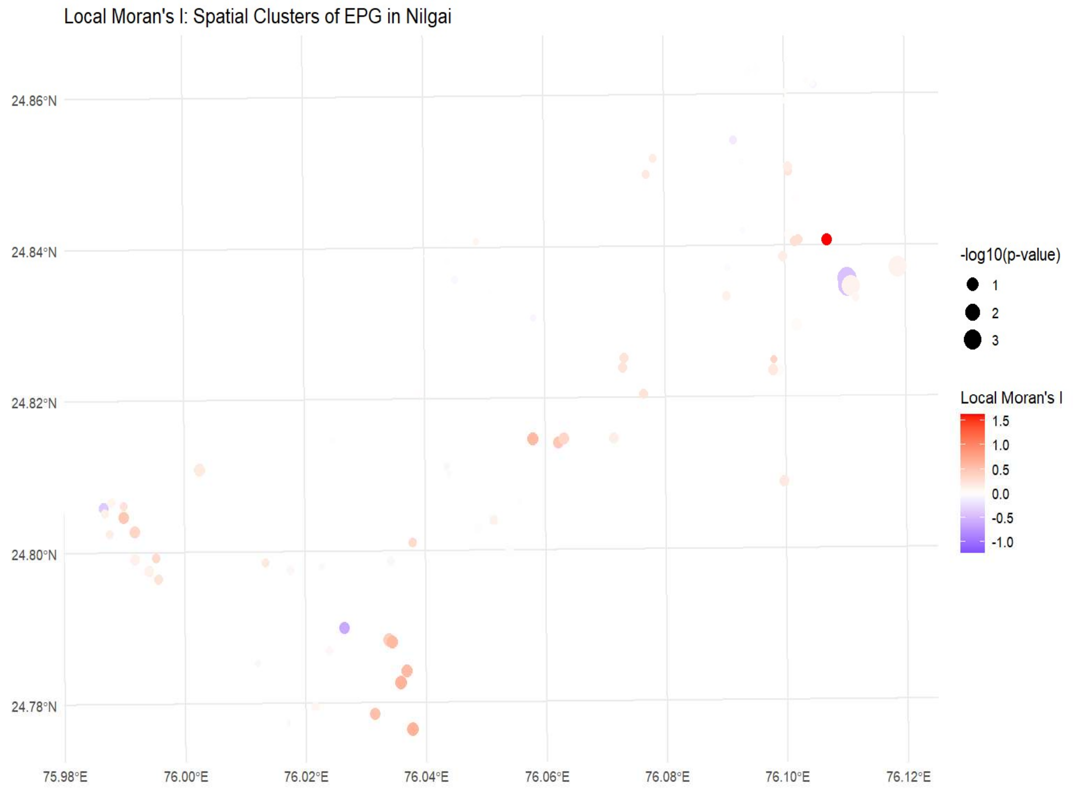

Figure 20: Spatial clusters of parasitic load in nilgai

Local Moran's I analysis was performed to examine the spatial clustering of EPG values in Nilgai. The results revealed one significant **high-high cluster**, indicated by the dark red dot in the eastern part of the study area (around 76.10°E, 24.84°N), where Nilgai with high EPG values were located near others with similarly high values. This suggests the presence of a localized hotspot of parasite load. Most other locations showed no statistically significant clustering, though some variation in Local Moran's I values was observed, represented by varying shades of red (positive autocorrelation) and blue (negative autocorrelation). The size of each dot represents the statistical significance of clustering, with larger dots indicating lower p-values (higher significance)

Hotspots (red points) represent locations with significantly high EPG values surrounded by other high values.

Coldspots (blue points) indicate locations with significantly low EPG values surrounded by other low values. The size of each point is proportional to the statistical significance of the cluster (-log10 p-value).

6.9: INFLUENCE OF SPATIOTEMPORAL OVERLAP ON NILGAI PARASITI LOAD

We examined the relationship between eggs per gram or EPG in Nilgai and their temporal overlap with cattle across different zones of the study area. EPG values were compared across two and three levels of cattle-Nilgai temporal overlap using non-parametric tests, due to the non-normal distribution of the data.

6.9.1 Temporal Overlap and Mean EPG Comparison

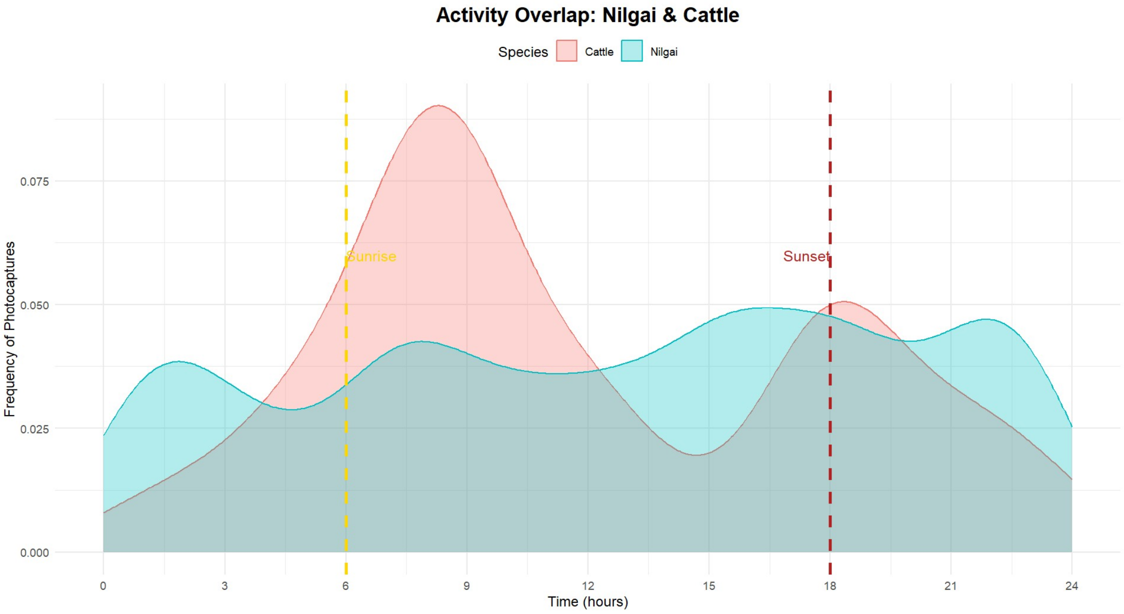

Figure 21: Temporal activity patterns and overlap of nilgai and cattle

Temporal Overlap Index

The overall temporal activity overlap (Δ) between Nilgai (*Boselaphus tragocamelus*) and domestic cattle (*Bos taurus*) was calculated as **0.693**, indicating substantial overlap in their daily activity patterns. This metric was derived using the **kernel density method (Δ_4 estimator)**, which quantifies the proportion of shared activity periods between two species within a 24-hour cycle.

The temporal overlap index (Δ) between Nilgai and cattle was calculated within each grid cell using a one-hour time window, providing a continuous measure of their diel co-occurrence.

To facilitate comparative analysis, particularly of eggs per gram (EPG) values across different overlap scenarios, the continuous Δ values were further categorized into discrete levels. In the two-level system, grids were classified as exhibiting either low overlap (Δ = 0), indicating no temporal co-occurrence, or high overlap (Δ > 0.0), representing any degree of shared activity. For a more nuanced analysis, a three-level categorization system was employed based on cattle presence and temporal overlap with Nilgai within each grid cell. The first category, "NA," included grids where cattle were absent, precluding any possibility of overlap. The second category, "No Overlap," comprised grids where both Nilgai and cattle were present but did not exhibit any temporal overlap within the designated one-hour window, indicating that their activities did not coincide. The third category, "Overlap," included grids where both species were present and demonstrated temporal overlap within the same one-hour window, reflecting simultaneous or near-simultaneous activity. Across all grids, observed Δ values ranged from 0 to 0.4, justifying the chosen category boundaries. This approach enabled systematic comparison of EPG values among grids with varying degrees of temporal overlap, thereby providing insights into the relationship between host co-occurrence and parasite transmission dynamics.

6.9.2: Descriptive Statistics for Nilgai EPG Across Spatiotemporal Overlap Groups

a. Two-category grouping

Temporal Overlap Group	n	Mean EPG	SD	Median	IQR
No Overlap (No cattle)	38	90.7	85.3	75	150
Overlap	58	146.5	113	150	137

Table 23: Descriptive statistics of nilgai parasitic load by temporal overlap with infected cattle (two groups)

Descriptive statistics were calculated for EPG in the two temporal overlap groups. The No Overlap group (n = 38) had a mean EPG of 90.8 (SD = 85.3), with a median of 75 and an interquartile range (IQR) of 150. The Overlap group (n = 58) had a mean EPG of 146.5 (SD = 113), with a median of 150 and an IQR of 137.

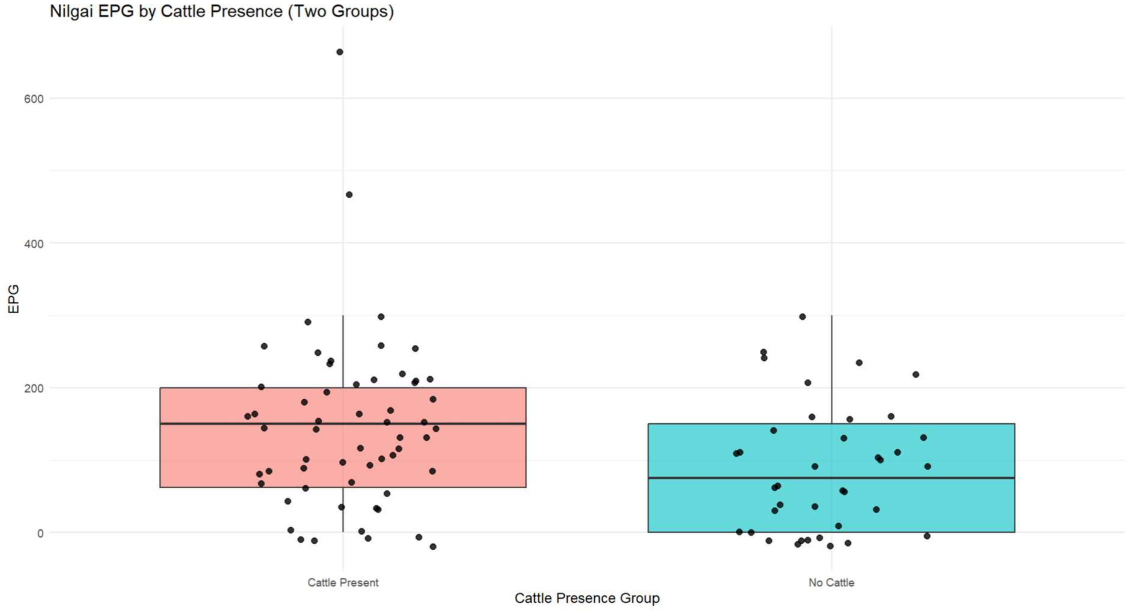

Figure 22: A box plot compares the distribution of Nilgai EPG across the Low and High temporal overlap groups.

b. Three-Group Comparison

Temporal Overlap Group	n	Mean EPG	SD	Median	IQR
No Cattle	38	90.8	85.3	75	150
No Overlap	3	200	50	200	50
Overlap	55	144	115	150	150

Table 24: Descriptive statistics of nilgai parasitic load by temporal overlap with infected cattle (two groups)

Descriptive statistics were calculated for EPG to summarize the central tendency and dispersion of values within each temporal overlap group. For the No Cattle group (n = 38), the mean EPG was 90.8 (SD = 85.3), with a median of 75 and an interquartile range (IQR) of 150. For the No Overlap group (n = 3), the mean EPG was 200.0 (SD = 50.0), with a median of 200 and an IQR of 50. For the Overlap group (n = 55), the mean EPG was 144 (SD = 115.0), with a median of 150 and an IQR of 150.

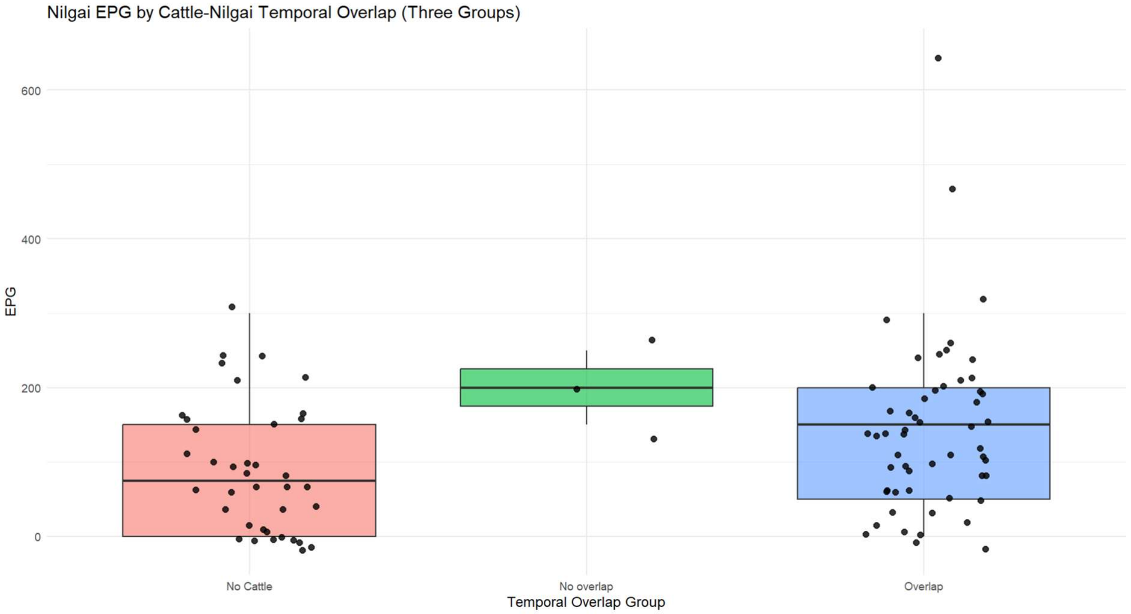

Figure 23 A boxplot visually compared the distribution of nilgai epg across the low, medium, and high temporal overlap groups

6.9.3: Distribution Normality for EPG Across Spatiotemporal Overlap Groups

a. Two Group

Temporal Overlap Group	Shapiro-Wilk p-value	Normality Assumption Met?
No Overlap	0.00102	No
Overlap	3.50×10^{-12}	No

Table 25: Distribution normality of nilgai epg across temporal overlap groups (Two-group)

b. Three Group

Temporal Overlap Group	Shapiro-Wilk p-value	Normality Assumption Met?
No Cattle	0.00102	No
No Overlap	1.97×10^{-9}	No
Overlap	0.0394	No

Table 26: Distribution normality of nilgai epg across temporal overlap groups (Three-group)

The assumption of normality for the egg per gram (EPG) values was assessed using the Shapiro-Wilk test for each temporal overlap group. The results indicated significant deviations from normality across all groups. For the three-group comparison, the p-values were 0.001 for the 'No Cattle' group, 1.97×10^{-9} for the 'No Overlap' group, and 0.039 for the 'Overlap' group. Similarly, for the two-group comparison, the p-values were 0.001 for the 'No Overlap' group and 3.5×10^{-12} for the 'Overlap' group. Since all p-values were below the threshold of 0.05, the null hypothesis of normal distribution was rejected for all groups. Therefore, non-parametric tests were deemed appropriate. The Kruskal-Wallis test was employed to compare EPG values across three temporal overlap groups, and the Wilcoxon rank-sum test was used for two-group comparisons.

6.9.4: Effect Of Spatiotemporal Overlap With Cattle On Nilgai Parasitic Load: Two-Group And Three-Group Comparisons And Correlation Analysis

Two-group Wilcoxon Rank Sum Test

Test Type	Grouping Used	Groups Compared	Test Statistic	df / W / S	p-value	Interpretation
Wilcoxon Rank Sum Test	2 categories	No Cattle vs Cattle Present	W = 756	-	0.008682	Significant difference in EPG

Table 27: Wilcoxon tests assessing the effect of temporal overlap with infected cattle on nilgai parasitic load

A Wilcoxon rank-sum test compared EPG values between nilgai with any temporal overlap with cattle (Overlap, n = 58, median = 150) and those with no cattle presence (No Overlap, n = 38, median = 75). The test revealed a significant difference, W = 756, p = 0.008, with higher EPG values observed in the Overlap group.

Three-Group Kruskal-Wallis Test

Test Type	Grouping Used	Groups Compared	Test Statistic	df / W / S	p-value	Interpretation
Kruskal-Wallis Test	3 categories	No Cattle, No Overlap, Overlap	χ^2 = 8.6739	df = 2	0.01308	Significant difference in EPG

Table 28: Kruskal- Wallis tests assessing the effect of temporal overlap with infected cattle on nilgai parasitic load

A Kruskal-Wallis test was conducted to compare EPG values among nilgai in three temporal overlap groups: No Cattle (n = 38), No Overlap (n = 3), and Overlap (n = 55). The test indicated a significant difference in EPG among the groups, $\chi^2(2) = 8.71$, p = 0.013. Median EPG values were 90 for the No Cattle group, and 200 for the No Overlap and 144 for Overlap groups. The highest mean EPG was observed in the No Overlap group (mean = 200), followed by the Overlap group (mean = 144), and the No Cattle group (mean = 90).

6.9.5. Correlation Between Overlap And Parasitic Load

A Spearman's rank correlation test was performed to assess whether a monotonic relationship exists between the continuous overlap values and EPG. The correlation was weak and statistically **non-significant** (*rho* = -0.119, *S* = 36385, *p* = 0.3727), indicating no consistent increase or decrease in parasite load with increasing overlap levels.

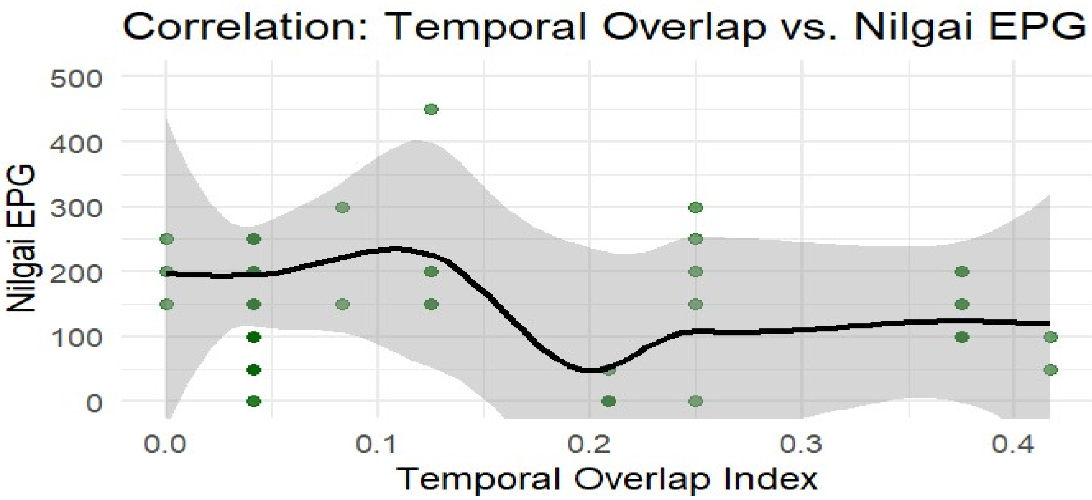

Figure 24: Correlation of temporal activity and nilgai parasitic load

7. DISCUSSION

The transmission of parasites and infectious diseases across the livestock–wildlife interface has become an increasingly important global issue, particularly in the wake of zoonotic outbreaks such as COVID-19, Nipah virus, and avian influenza. These events have brought important issues to the surface, like the necessity of keeping our heath of ecosystem in check, whether it be just for the humans and their pets/livestock, per se. We are all aware of the COVID-19 outbreak aftermath, it has become increasingly necessary to continuously monitor the health of ecosystems, where close interactions between humans, livestock, and wildlife occur, as such ecosystems can facilitate the emergence and spillover of novel pathogens. Unlike microbial disease, parasitic infections are often overlooked, as the latter do not lead to the rapid onset of symptoms or immediate mortality. In the ecosystem, livestock-wildlife zones heighten the cross-species transmission. The transmission risk becomes even more threatening when the potential for the disease to enter the human population increases. This study looked into the spatial and temporal relationships between nilgai (Boselaphus tragocamelus) and domestic cattle in a semi-arid landscape of Kota and tried to understand the influence on the dynamics of endoparasitic intensity and prevalence in nilgai and cattle across the gradients of temporal overlaps and the spatial proximity overlap. Our findings indicate that spatial proximity and temporal overlap with cattle are significantly associated with increased parasite load and infection prevalence in nilgai, supporting the hypothesis that livestock-wildlife interfaces facilitate parasite transmission. To understand the spatial and temporal pattern of parasite burden and prevalence on nilgai first step was to understand the spatial extent of habitat use. Space used by nilgai was 6,838.77 hectares (68.39 km^2), while that of cattle was 6,129.73 hectares (61.30 km^2). Notably, the area of overlap between the two species was substantial, measuring approximately 56.08 km^2. Large, noticeable spatial overlap suggests that species are utilizing similar habitats within the enclosure, and thus, the potential of disease transmission could be high. There are several studies conducted across the world that state the movement and spatial overlap of wildlife and livestock can lead to an increased risk of disease transmission. Areas such as forest edges, cropland, pastureland, grassland, and water resources are hotspots for disease transmission (Titcomb et al., 2023).

A Kruskal-Wallis test revealed a significant difference in the EPG values between nilgai located near cattle within 1000 meters compared to those further away. This similar pattern remains persistent even across the finer spatial overlap unit. The Kruskal-Wallis test revealed a significant difference in EPG values among the nilgai grouped by proximity to cattle (High: ≤1,000 m; Medium: 1,000–2,000 m; Low: >2,000 m). Post hoc pairwise comparisons using the Wilcoxon rank-sum test with Benjamini-Hochberg correction revealed statistically significant differences in EPG values across all proximity groups. This test indicates that the parasitic load varied consistently with livestock proximity. Statistically, individuals in high proximity zones had significantly different EPG values compared to those in medium and low zones ($p = 0.0081$), and even medium proximity differed significantly from low ($p = 0.0095$). These findings suggest that there is an increasing gradient of parasite exposure in nilgai associated with closer contact with livestock. The presence of gradiation supports the idea that the livestock-wildlife interface may facilitate parasite transmission to wild ungulates. Many gastrointestinal nematodes infecting both livestock and wild ungulates have life cycles that include free-living stages in the environment. After being shed in the feces, parasite eggs or larvae develop on pasture and can survive for days to weeks. Both nilgai and cattle grazing in the same areas—even if not simultaneously—can ingest these infective larvae from contaminated vegetation. This environmental reservoir means that spatial overlap in grazing areas directly increases the risk of cross-species parasite transmission, as infective stages are available to any grazing animal. Thus, the observed association between proximity to cattle and higher parasite prevalence in nilgai is likely driven by this mechanism of environmental cross-contamination.

This similar gradient was even more pronounced when the influencing variable was changed to the proximity to infected cattle; before that, we used cattle presence proximity. This approach (Proximity to infected cattle) was taken cause the presence of cattle in space and time does not necessarily have to have disease/parasite, especially when the feral cattle becomes part of the natural ecosystem. With this approach, we knew for sure that the probability of a nilgai being infected is higher when it is close to infected cattle, giving us an ecologically accurate picture of parasite transmission dynamics.

The Wilcoxon rank sum test revealed that Nilgai located closer to infected cattle (“High” proximity group) has significantly higher EPG values compared to those farther away, as indicated by the Wilcoxon rank sum test ($W = 1982.5$, $p < 0.001$). This relationship was further supported by the Kruskal-Wallis test, which revealed significant differences in EPG distributions across all proximity groups ($\chi^2 = 42.20$, $df = 2$, $p < 0.001$). Post hoc pairwise comparisons clarified the nature of these differences: both the High vs. Medium and High vs. Low groups differed significantly ($p < 0.001$), while there was no significant difference between the Medium and Low groups ($p = 0.3$). That elevated parasite burdens in nilgai are most pronounced in individuals near infected cattle, with the effect diminishing at greater distances. Together, these results highlight that both the presence of cattle and the infection status of cattle play a significant role in determining the parasite burden in nilgai. The presence of cattle elevates the risk of parasite transmission, while the presence of infected cattle amplifies this effect, creating a hotspot of transmission.A conceptual synthesis by Altizer et al. (2021) states that spatial distance, genetic similarity, and temporal or ecological niche overlap together define a gradient of transmission risk for parasites. He uses the term "generalized transmission distance" for these factors.

The paper explains that proximity among the animals, genetic similarity, and how much their activities or habitats overlap—all these factors together create a gradient (or range) of transmission risk for parasites. Within a group, very close individuals (high overlap) have high risk, while animals from different groups that are farther apart (low overlap) have low risk.The study done at the Yellowstone Wolf Project reported that *Toxoplasma gondii* infection in Yellowstone wolves is strongly predicted by the degree of spatial overlap between wolf territories and cougar-dense areas(Meyer et al., 2022). This spatial pattern is consistent with the previous research, indicating that livestock act as reservoirs for parasites, and the risk to wildlife health is at its highest at the high overlap zones and with livestock having high infection prevalence. (Bagchi et al., 2004; Chakraborty et al., 2019).

Spatial Correlates of Parasite Prevalence and Intensity in Nilgai

The Previous studies have shown that parasite prevalence and its intensity in wild ungulates often resemble those of nearby livestock. This influence is even more pronounced in areas of high overlap (Chakraborty et al., 2019; Ezenwa, 2003). But it's not just cattle that could be a potential source of spillover; shared resources equally act as a major source of origin. To understand the association of risk factors such as water hole and village point with Nilgai parasite prevalence and intensity (measured as EPG), Spearman correlation analysis was done; the result revealed a significant negative correlation of nilgai parasite prevalence (rho = -0.354, $p < 0.001$) and intensity (rho = -0.484, $p < 0.001$) with that of distance from cattle These result indicates that nilgai closer to cattle are more likely to be infected and to carry higher parasite burdens.

But interestingly, nilgai parasite prevalence (rho = 0.299, p = 0.003) and intensity (rho = 0.258, p = 0.011) were significantly positively correlated with distance to water, further supporting the hypothesis that environmental contamination with infective parasite stages is highest near cattle congregations, increasing exposure risk for nilgai at proximity to that environmental contaminant (Morgan et al., 2006). This pattern may reflect that areas like Kota Falls in semi-arid areas, where scarcity of water is a major issue, become a constraining issue for livestock and wildlife, thus forcing both wildlife and livestock to congregate around limited water sources, leading to increased environmental contamination and higher transmission risk in more distant areas. The result is supported by the findings of Hall et al. (2021), who demonstrated that water resource scarcity in dry or arid regions causes animals to congregate, which increases the fecal-oral parasite load in the environment. This effect gets amplified as water becomes more and more limiting. Thus, reduced resource availability leads to animal aggregation, and this results in a higher transmission risk (Titcomb et al., 2023).

No significant correlation was found between distance to village and parasite prevalence (rho = -0.126, p = 0.223) or intensity (rho = -0.105, p = 0.307). In contrast, other studies have reported increased parasite loads as animals move closer to villages or decreased loads with increasing distance, likely due to greater exposure to contaminated environments near human settlements (Wells 1999; Johns et al. 2004). It may be due to the enclosure boundary acting as a barrier to movement and potential transmission. Physical barriers such as fences can act as effective obstacles to wildlife movement, thereby reducing the risk of disease transmission between populations (Woodroffe et al., 2019). Previous studies have highlighted the role of water points as hotspots for parasite transmission due to the aggregation of multiple host species (Turner et al., 2013). This suggests that, within the study landscape, proximity to village settlements does not directly influence the risk of gastrointestinal parasite infection in nilgai.

Spatial Correlates of Parasite Prevalence and Intensity in Cattle

Unlike Nilgai, the Spearman correlation analysis result of parasite prevalence and intensity in cattle showed no significant association with distance to village or distance to water. Both variables showed only weak or very weak correlations with cattle parasite metrics. Distance to village had a weak, negative correlation with cattle prevalence (ρ = -0.206, p = 0.065) and EPG (ρ = -0.139, p = 0.215), while distance to water showed a very weak, positive correlation with both prevalence (ρ = 0.053, p = 0.636) and EPG (ρ = 0.063, p = 0.574). The lack of significant spatial correlations suggests that cattle parasites are uniformly distributed across the study area and are less influenced by proximity to villages or water sources.

Comparison with Nilgai Findings

Influence of environmental variables (Distance to water and Distance to village) on cattle parasitic intensity and prevalence contrast with the patterns observed in nilgai, where both parasites. The lack of significant spatial correlations in cattle could be attributed to the ecology of cattle. Unlike other wild ungulates, Cattle move actively in big herds, either alone or by herders, or likely due to the herding behaviour of livestock. They graze together in a single group and aggregate at the water point, constantly exposed to similar parasite burdens. Unlike nilgai, cattle are not selective foragers or do not show any territorial or home range behavior, and they move freely across the landscape and utilize a broader range of habitats and water resources, thus increasing the cross-transmission of parasite, From field observations, all the cattle would congregate in the evening at one location next to the enclosure boundary, which is close to villages. The next morning, they would spread out for foraging and then return in groups, numbering in the hundreds. This behaviour masks or dilutes the parasitic prevalence or load across the population. Whereas this pattern was visible in nilgai hotspot map (fig: 6.16). Where we are more likely to encounter a localized hotspot of infection, particulary the individuals that are close to high cattle density areas, Another reason is unlike cattle, nilgai show territorial behaviour and are selective foragers, this restricts the indviduls movement to limited space thus reducing the chances of dilution in the populations. Nilagi in semi-arid areas can be seen in herds ranging from 2 to 8, rarely 10. The occurrence of small herd size becomes more frequent when the resources are depleted and non uniformly distributed. This could be the reason why there are small but numerous parasite prevalence hotspot areas (Fig: 6.16) of nilgai distributed across the landscape. Whereas cattle have few but large hotspot zones (Fig : 6.15).

The spatial analysis of EPG (eggs per gram) values in nilgai using both Global and Local Moran's I provides intriguing evidence for the presence of spatial clustering in parasite infection intensity across the study landscape. The significant positive Global Moran's I value (Moran's I = 0.12, expected = -0.01, variance = 0.0025, standard deviation = 2.66, p = 0.0039) indicates that similar EPG values are more likely to be found near each other than would be expected under a random spatial distribution. In practical terms, this means that high (or low) parasite burdens in nilgai are not randomly scattered, but instead tend to form spatial clusters.

The Local Moran's I analysis further refines this understanding by pinpointing the exact locations of these clusters. The size of each point on the map is proportional to the statistical significance of the cluster (-log10 p-value), highlighting which clusters are most robust.

The presence of both hotspots and coldspots underscores the heterogeneity of parasite risk within the landscape, which has important implications for targeted management and surveillance. In Figure 6.17, cold spots (areas of significantly low EPG values) are typically located farther from water points. If we look at the association of Hotspots, Coldspots with the Villages, neither Hotspots nor Coldspots are strongly associated with village locations. Hotspots are not clustered near villages, and coldspots are not consistently found at greater distances from villages. This observation aligns with the correlation analysis, which found no significant relationship between distance to village and nilgai parasite prevalence or intensity. This suggests that, in this landscape, human settlements do not directly drive the spatial clustering of parasite infection in nilgai. Additionally, Both the two-group and three-group analyses consistently demonstrate that nilgai in areas with cattle—regardless of whether they overlap in time—have higher gastrointestinal parasite loads than nilgai in areas without cattle. This suggests that cattle presence, and possibly shared resources or environments, may facilitate parasite transmission to nilgai

This pattern is consistent with studies from Spain and Africa, which reported that when wild and domestic ungulates use the same areas at similar times—especially around key resources like water points—there is a higher incidence of diseases such as bovine tuberculosis in both groups. This demonstrates that spatiotemporal overlap between wildlife and livestock increases the risk of cross-species disease transmission (Barasona et al., 2016).

These results reinforce the importance of considering not only where, but also when, wildlife and livestock interact when assessing disease risk and designing targeted management interventions. For example, Oliveira-Santos et al. (2023) found that predator-induced segregation between deer and moose reduced parasite spillover risk, while increased overlap would elevate transmission risk. Similarly, Wang et al. (2023) observed that higher spatiotemporal overlap between coyotes and dogs was associated with increased prevalence of Echinococcus multilocularis. Chang et al. (2020) also discuss how increased spatial and temporal overlap among hosts and parasite stages raises transmission risk. Multiple studies further describe how parasites can alter animal behavior to increase transmission rates (Poulin, 2010). Thus, spatiotemporal overlap is a key driver of parasite transmission and burden in wildlife populations (Craft, 2015).

8. Management Implications

This study was conducted in the Darra enclosure, a landscape designated for rewilding. The area supports both feral livestock that reside permanently within the enclosure and domesticated cattle that are intentionally released by local villagers, often due to chronic fodder shortages. In Rajasthan and other semi-arid regions, livestock are a vital livelihood resource, and dependence on forest grazing increases during periods of scarcity. Because cattle are relatively easy to manage and are a primary source of parasite introduction, their movement and health status are especially important for disease management.

Given these realities, practical management must balance conservation goals with the needs of livestock-dependent communities. The findings highlight the substantial spatial overlap, driven by shared grazing lands and water sources, which demonstrates the permeability of boundaries between livestock and wildlife in human-modified landscapes. This overlap, particularly at water points, facilitates indirect environmental transmission and is a key mechanism for cross-species parasite exchange. Many gastrointestinal nematodes, such as strongyles, have free-living larval stages that develop on pasture after being deposited in feces, enabling infection of any herbivore grazing the area, regardless of whether cattle and nilgai are present simultaneously (Sharma et al., 2017; Shirbhate et al., 2022).

Because domestic livestock can enter the enclosure and represent a primary source of environmental contamination, strategic deworming is essential to reduce the introduction and spread of infective parasite stages (Wiese et al., 2021; ATTRA, n.d.). However, simply deworming animals and immediately moving them to clean areas is insufficient, as viable parasite eggs can still be shed for a period after treatment. Best practice involves holding livestock in their original location for 12–24 hours post-deworming before moving them, which helps minimize pasture contamination and reinfection risk (ATTRA, n.d.). In addition to regular deworming, close management of livestock movement is necessary. This includes restricting or monitoring entry points and ensuring that livestock are not returned to heavily contaminated areas.

Rotating livestock through different grazing areas helps break parasite life cycles and prevents overgrazing (Waller, 2006; Sutherland & Scott, 2010). Increasing the number of water points and distributing them more widely across the landscape can reduce the congregation of animals at any single site, thereby lowering the risk of environmental contamination and cross-species parasite transmission (Turner et al., 2010; Kock et al., 1999). Increasing the number of water points and distributing them more widely across the landscape can reduce the congregation of animals at any single site, thereby lowering the risk of environmental contamination and cross-species parasite transmission (Turner et al., 2010; Kock et al., 1999). In addition, maintaining or restoring aquatic vegetation around water bodies is important, as reduced plant cover has been linked to higher parasite loads in water, increasing infection risks for wildlife such as red deer. Aquatic plants act as natural filters and barriers, limiting the survival and transmission of certain parasite stages in shared water sources (Rysava et al., 2016)**.** Maintaining taller grasses and actively planting grass in overgrazed areas are also important. Parasite larvae are typically found in the lower 1–2 inches of vegetation, so encouraging livestock to graze higher by maintaining taller swards reduces the likelihood of ingesting infective larvae (Sutherland & Scott, 2010; Kaplan & Vidyashankar, 2012). Restoring vegetation in heavily grazed patches not only improves pasture quality but also serves as a natural barrier to parasite transmission (Waller, 2006).

These combined strategies—rotational grazing, increasing and distributing water and feeding points, and pasture restoration—have been adopted in various rangeland and conservation projects to successfully reduce parasite loads and improve both livestock and wildlife health. Additionally, focusing interventions near water sources and high-density livestock areas can further enhance effectiveness (Shirbhate et al., 2022).

Organizing regular, coordinated deworming of both feral and domestic cattle—with the involvement of local veterinary services and village committees—can significantly reduce environmental parasite loads, especially when treatments are timed before peak grazing periods or seasonal migrations (Terefe et al., 2023). Collaborating with local communities to implement rotational grazing plans allows pastures and forest patches to rest and recover, which helps break parasite life cycles. Where total exclusion is not feasible, temporary or seasonal restrictions on livestock entry to sensitive areas such as water points or wildlife hotspots can still be effective (Terefe et al., 2023). Outreach programs that highlight the benefits of parasite control for both livestock productivity and wildlife health, along with incentive schemes like fodder support, veterinary care, or grazing rights, can encourage community participation in these management efforts.

Importantly, proximity to villages was not a significant predictor of parasite burden in nilgai, likely due to management barriers such as the enclosure wall, which limit wildlife movement toward settlements and livestock toward the forest area. To ensure continued effectiveness, regular monitoring and maintenance of these barriers are recommended to prevent potential breaches or infiltration. This suggests that, in rewilding landscapes, interventions focused on natural resource points, such as waterholes and shared grazing lands, may be more effective than those targeting the village–wildlife edge.

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