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Research Article

Investigation of enterotoxaemia outbreaks in Chinkara Deer (*Gazella bennettii*) kept under tropical climatic conditions

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ABSTRACT

The current study recorded clinic-pathologic data during an outbreak of enterotoxaemia in Chinkara deer (*Gazella bennettii*), which led to the death of thirteen of the thirty-four animals. Young animals had a significantly ($P<0.05$) higher death rate than older animals. The clinical signs included severe loss of appetite, significant lethargy, animals huddling in a corner, elevated body temperature, urine with a dark brown color like chocolate, and diarrhea with a greenish-watery consistency. The postmortem examination of the dead deer showed the existence of a straw-colored exudation in the peritoneal cavity. The affected animals consistently exhibited congested and edematous lungs, hydropericardium, hydrothorax, watery contents in the small intestine, bloated and soft kidneys, opisthotonus, and ballooning and hemorrhages of the small and large intestines. The myocardium and jejunal mucosa were frequently the sites of multifocal petechial hemorrhages in acute cases. Pulmonary edema, perivascular cuffing in the lungs, congestion, proteinaceous fluid in the alveoli, and hemorrhagic enteritis were the most significant histologic changes. Thirteen deer were necropsied, and the urine from their bladders showed a high glucose content.

The renal tubular epithelial cells were disrupted, and necrosis and congestion were observed during the histopathological examination of the kidneys. The clinical manifestations, post-mortem lesions, histological results, and isolation were all compatible with enterotoxaemia caused by *C. perfringens* type D being the root cause of mortality.

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Introduction

Clostridium perfringens is a rod-shaped, spore-producing, anaerobic, and gram-positive bacteria of wild as well as domestic animals (Bennett et al. 2019). In small ruminants, Enterotoxaemia is a significant and widespread disease, with a 40% prevalence rate in Pakistan (Hussain et al. 2018). This disease primarily affects ovine and caprine species, with an infrequent incidence in larger animals (Ahmad et al. 2015). The peracute disease often affects young, adult, and non-vaccinated sheep and goats (Zafar Khan et al. 2022). Enterotoxaemia is a condition that is caused by the bacterium *Clostridium perfringens* type D and is found all over the world (Hussain et al. 2022). The reason for sudden deaths is attributed to the presence of four primary toxins, specifically beta, iota, epsilon, and alpha, which are generated by the

bacteria *C. perfringens* (Uzal et al. 2014). Death results from toxemia, which occurs when the small intestinal tract releases toxins into the circulation, which subsequently travel to internal organs and damage them (Khan et al. 2022). The prevalence of the condition is greatly affected by excessive consumption of lots of grain and forage, abrupt dietary changes, and the small intestine's deficiency in oxygen environment (Riaz Hussain et al. 2014). Clostridium perfringens type D spores are frequently present in both feces and soil (Revitt-Mills et al. 2015). Even though *C. perfringens* usually live in the intestine, their population is usually quite low. These bacteria normally create trace levels of toxins, which are either neutralized by circulating antibodies or removed by gastrointestinal motility (Bhoyar). Lethal infections often arise as a result of predisposing conditions that facilitate the bacteria's rapid proliferation in the gastrointestinal tract (Uzal et al. 2014). Young animals are predominantly affected by acute disease, because the organisms reproduce quickly and produce toxin at a rate that surpasses the body's ability to clear or neutralize them. Pulpy kidney disease is the term used to describe enterotoxemia in sheep caused by the bacterium Clostridium perfringens type D (Ahmad et al. 2015). Different types of lesions seen in this infection include enlarged and soft kidneys, large and small intestinal hemorrhages, fluid surrounding the heart and lungs (hydropericardium and hydrothorax, respectively), and serosal and muscle hemorrhages in the colon and heart (Riaz Hussain et al. 2014) (Uzal et al. 2014). The essential resources for establishing a preliminary diagnosis include history, clinical presentation, macroscopic lesions found during necropsy, and histological findings (Sessa *et al.*, 2020). There are few descriptions of gross clinical and microscopic abnormalities seen in spontaneously occurring cases of *C. perfringens* type D enterotoxemia in Chinkara deer (*Gazella bennettii*) (Khan et al. 2022). The current investigation describes the clinical picture, gross, and histological results in 13 Chinkara deer suspected of having passed away from enterotoxaemia.

Materials and Methods

The present study was carried out during an outbreak of enterotoxemia in Chinkara deer (*Gazella bennettii*) in Bahawalpur district, Punjab, Pakistan. The ecological temperature in that area ranged from 41.3°C to 42.5°C, with 63.1%. relative humidity. A flock of 34 Chinkara deer (*Gazella bennettii*) were examined, from which 16 Chinkara deer were brought from Bahawalpur Zoo and 18 from IUB. The age of these deer was one to three years. 13 chinkara deer out of 34 had died. These deer were not vaccinated against any diseases. They were fed lush green feedstuff, specifically Barseem (*Trifolium alexandrinum*), and provided with ad libitum fresh drinking water.

Clinical observations

The clinical manifestations observed in effected animals included moderate to acute anorexia, head down in a corner, severe depression, fever, chocolate-colored urination, and passing of greenish watery diarrhea. Treatment involved oral administration of antibiotics such as Amoxicillin (10mg/kg), Tribersen (15mg/kg), and chlortetracycline-20 (10 mg/kg). Additionally, a vit-mineral mixture (1 ml/Lin drinking water) was provided to the affected deer. Despite treatment, the affected animals did not indicate any improvement towards recovery as clinical signs remain, and mortality occurred.

Histopathological investigations

Necropsies were carried out within one hour of death, and considerable changes were discovered. Morbid tissues from the airways, kidneys, liver, and gut were collected and stored in a 10% neutral-buffered formalin solution for fifteen days. Fixation, dehydration, infiltration, and embedding were Standard histopathological procedures used. The preserved tissues were sliced into four microns thick sections and using the Hematoxylin and Eosin staining (Li et al. 2018)

Cultural and colony morphological features

Samples for bacterial culture were taken aseptically from the kidneys, lungs, heart, and small and large intestines. After collection, these samples were promptly kept at 4°C and grown anaerobically and aerobically for 2-3 hours on blood agar medium containing 5% deer blood. The cultured growth was then stained on a microscopic slide with Gram stain, displaying gram-positive rods using a 100x objective lens.

Statistical analyses

Chi square analyses was performed on the collected data with a significance threshold of $P \leq 0.05$. The odd ratios and confidence intervals of 95% were also calculated.

Results and Discussion

The current examinations were conducted on 13 dead Chinkara deer (*Gazella bennettii*) during an enterotoxaemia outbreak. Aerobically incubated samples yielded little growth. Out of 44 animals, death was recorded in 13 Chinkara deers. Enterotoxaemia affects 40% of sheep in Pakistan (Mohiuddin et al. 2020). Enterotoxaemia-related deaths in sheep, goats, and calves have already been recorded (Riaz Hussain et al. 2014). In our investigation, the death rate varied between 10 (20%; 95% CI = 10.63 - 32.76) and 35 (33.3%; 95% CI = 24.82 - 42.75) in two deer flocks. The study found no significant difference in mortality rates between male and female deer. However, mortality rates were considerably greater ($P<0.05$) in deer

under one-year-old compared to those over one (Table 2).

Effects on lungs

Histopathological observations of lungs, deer mortality occur due to enterotoxaemia showed that the lungs were extremely congested, darker, edematous, inflated, and showed consolidation. Atelectasis (collapse of part or all of the lung), Emphysema (chronic obstructive pulmonary diseases), hemorrhages, and interstitial pneumonia were also observed.

Table 1: Different pathological lesions (gross lesions) recorded in chinkara deer (n=13) died of enterotoxaemia

Gross lesions	Frequency		95% CI
	No	%	
Hydrothorax	9	69.23	41.30 - 89.37
Hydroperitonium	11	84.61	57.77 - 97.34
Hydropericardium	8	61.53	34.09 - 84.32
Petechial hemorrhages on myocardium	7	53.84	27.43 - 78.70
Congested lungs	9	69.23	41.30 - 89.37
Pulmonary edema	10	76.92	49.14 - 93.77
Swollen and soft kidneys	11	84.61	57.77 - 97.34
Hemorrhages on kidneys	12	92.30	67.52 - 99.62
Watery contents in intestine	11	84.61	57.77 - 97.34
Hemorrhages on jejunal mucosa	9	69.23	41.30 - 89.37
Ballooning and hemorrhages of small intestine	11	84.61	57.77 - 97.34

Table 2: Overall mortality (%age) recorded in deer died of enterotoxaemia

Sex/age	No. of Animal	Mortality		95% CI	Odd Ratio/ P value
		N	**%**		
Sex					
Female	15	5	33.33	13.38 - 59.21	OR = 1.45 [reciprocal = 0.69]
Male	19	8	42.10	21.83 - 64.63	
Age groups					
< 1Year	12	5	41.66	17.17 - 69.79	Mantel-Haenszel chi-sq P = 0.794
1-2 Year	11	4	36.36	12.79 - 66.36	
3 Years	11	4	36.36	12.79 - 66.36	

Effects on liver

Hepatocytes with eccentric nuclei, degeneration of hepatocyte, karyorrhexis, nuclear hypertrophy, pyknosis, hemorrhages, vacuolar degeneration, ceroid formation, congestion observed in deer died as a result of enterotoxaemia.

Effects on kidney

The kidneys were found to be highly consolidated and darker in color, with significant edema, deterioration of glomerular congestion, and hyperemia. There were scattered hemorrhages on both the mucosal and serosal surfaces. Deer that died from enterotoxaemia had expanded bowman's space, ceroid development, tubular cell necrosis, melanomacrophage aggregate, nuclear hypertrophy, degeneration and obliteration of renal tubule.

Effects on small intestine

The histopathology of intestinal sections indicated degeneration, villi fusion and necrosis, congestion, edema, and bleeding in the lamina propria and submucosa. Inflammatory cells invaded the mucosa and submucosa of both the small and large intestines.

Discussion

High death rates in young animals have already been recorded in other animal species (Uzal et al. 2014). History indicates that the subtropical climate of Pakistan's Punjab province has a major impact on disease. Per acute and acute deaths have been reported in Chinkara deer (Javed et al. 2009), cattle and sheep (Hussain et al. 2018) previously, under similar conditions. In our investigation, all infected animals in acute cases displayed a variety of clinical symptoms, such as moderate to acute anorexia, head down in corner, fever, severe depression, opisthotonus, chocolate colored urine, and greenish watery diarrhea. However, in cases where animals died from a per acute infection, no clinical symptoms were seen. Clinical indicators of acute enterotoxaemia include rapid breathing, foaming at the mouth, opisthotonus in deer, and profuse, abdominal pain, greenish-watery diarrhea, discomfort, and other neurological signs of colonic convulsion. (Riaz et al. 2019), cattle (Karthik et al. 2017), sheep and goats have been reported (Javed et al. 2009). The clinical signs seen in this

investigation have also been documented in cattle. (Riaz Hussain et al. 2014), sheep, lambs and goats (Naz et al. 2012).

Extreme amounts of straw-colored, proteinaceous fluid were found in the thoracic, abdominal, and epicardium cavities of dead animals, both in acute and peracute cases, according to necropsy data. In Pakistan, reports of similar necropsy outcomes have been made for deer, sheep, and goats (Javed et al. 2009). The kidneys of affected animals were darker in color and exhibited signs of congestion, softness, and swelling. Similar gross changes in the lungs and kidneys have been reported in cases of naturally and experimentally induced enterotoxemia in deer (Khan et al. 2018), goat and sheep (Uzal et al. 2014). During postmortem examination, lungs in all animals exhibiting respiratory signs were congested, edematous, and consolidated. Petechial hemorrhages were observed in the myocardium, jejunal mucosa and at the base of the heart. In deer during acute episodes, ballooning and hemorrhages of lower Gastrointestinal tract were often observed, along with the presence of greenish watery material as mentioned by (Ali et al. 2024). The study's gross lesions are comparable to those seen in past enterotoxemia cases involving deer, cattle, and other small ruminants (Mohiuddin 2016). The lungs displayed notable changes under the microscope, including inflammatory material and diffusely packed eosinophilic proteinaceous edema fluid throughout the bronchi, bronchioles, and bronchi. The lung tissue showed thickening and a significant inflammatory cell infiltration of the bronchioles. The lungs were also heavily infiltrated with leukocytes, especially mononuclear cells, and were congested. There have been earlier reports of such profound microscopic alterations in deer (Khan et al. 2018), lamb (Omer et al. 2020) cow, calf, sheep and goats (Pawaiya et al. 2020). Histologically, the liver showed significant congestion and cytoplasmic vacuolation of hepatocytes. The intestinal histological changes that were most noticeable were necrosis, hemorrhagic enteritis, villus epithelium loss, enlargement of intercellular gaps, and congestion. Similar intestinal changes in deer have been observed as a result of enterotoxaemia (Renu et al. 2021), cattle, calves, sheep, goat (Nazki et al. 2017) and in rabbits (Solans et al. 2019). The glomeruli were inflamed, there were large interstitial hemorrhages, and pyknosis (shrinkage and increased basophilia of the nucleus) and necrosis of renal tubular cells were the predominant degenerative alterations in the kidney. Similarly, histopathological lesions were reported in the kidneys of sheep and goats (Javed et al. 2009). The changes observed in the kidneys of affected deer included greater urinary spaces, glomeruli atrophy, and sloughing of renal epithelium. There have been reports of similar pathological changes in the kidneys of several animals dying from enterotoxaemia (Mohiuddin 2016). The elevated urine glucose level of dead deer was also observed. Elevated glucose levels in urine are considered as a typical sign of enterotoxemia in sheep (Uzal et al. 2014). Based on the findings, it can be said that enterotoxemia in deer bears similarities with sheep, goats, and other species of animals in terms of pathophysiology, clinical manifestations, and histological indicators (Attia; Jemal et al. 2016). The present study suggests a strong correlation between overeating of lush green fodder and sudden fluctuations in environment with the occurrence of enterotoxemia**.**

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Ethical Statement

No Ethical permissions were required for this article.

Availability of Data and Material

The data can be obtained from the corresponding author on a reasonable request.

Consent to Participate

All the authors gave their consent for equal participation.

Consent for Publication

All the authors gave their consent for publication.

Competing Interest

The authors declare that they have no relevant financial or non-financial interests to disclose.

Author Contribution

All the authors contributed equally in this manuscript.

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