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REVIEW

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A review of clostridial diseases in rhinoceroses

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Abstract

Although rhinoceros conservation efforts are focused on anti-poaching and combating wildlife trade, infectious diseases are an increasing threat to both free-ranging and captive rhinoceros populations. Among these, clostridial infections with *Clostridium perfringens*, *Paeniclostridium sordellii*, and *Clostridium novyi* have caused acute disease in black and white rhinoceroses, characterized by a high mortality rate within very brief time spans. The acute nature, as well as limited knowledge of epidemiology, diagnosis, and management, make clostridial diseases an important threat to rhinoceros conservation. This article reviews historical cases of clostridial disease in rhinoceros with the aim of formulating a clearer understanding of the disease's epidemiology and using this information to make recommendations for its management in free-ranging rhinoceroses. Acute colic and sudden death in rhinoceroses, together with necropsy findings of segmental hemorrhagic enteritis, should lead towards a tentative diagnosis of enteric clostridial disease, although detection and identification of specific toxins are required for a definitive diagnosis. Our findings indicate the need for further research into clostridial disease dynamics in wildlife species and provide the basis for this research in rhinoceroses.

KEYWORDS

clostridial disease, infectious disease, rhinoceros, wildlife health

1 | INTRODUCTION

The decline of rhinoceros populations is one of the most significant tragedies that has faced wildlife (Dublin & Wilson, 1998). Although poaching represents the greatest threat to rhinoceros conservation, health-related conditions are also capable of causing high mortalities and lowering individual reproductive potential and therefore population growth rates (Emslie & Brooks, 1999; Khan & van Strien, 1997). Notable infectious diseases impacting rhinoceros conservation globally include bovine tuberculosis, salmonellosis, trypanosomiasis, and clostridial diseases (Dwyer et al., 2022; Jonyo, 1989; Miller et al., 2018; Ramsay & Zainuddin, 1993).

Clostridium species are ubiquitous in the environment, inhabiting soils (as vegetative cells or highly resistant spores) and as commensals in the intestinal tracts of domestic and wild animals (Songer, 1997). Infections with these Gram-positive spore-forming anaerobic bacteria can lead to an array of clinical disorders, depending on the clostridial species involved. Pathogenic species that cause disease include *Clostridium botulinum*

(botulism), *Clostridium tetani* (tetanus), *Clostridium perfringens* (enterotoxaemia), *Clostridium novyi* (wound infections and infectious necrotic hepatitis), *Clostridium chauvoei* (blackleg), *Clostridium difficile*, *Clostridium septicum* (malignant edema), and *Paeniclostridium sordellii* (toxic shock syndrome) (Uzal et al., 2016). Toxins produced by different species have selective affinity for the nervous system (neurotoxic), tissues (histotoxic), and intestines (enterotoxic) (Lewis, 2007).

Clostridial diseases are a substantial problem for both domestic animal and wildlife managers due to (1) the ubiquitous distribution of these bacteria; (2) limited knowledge of predisposing factors for toxin production and disease; (3) the acute nature of disease, often manifesting as sudden death; and (4) difficulty in obtaining a diagnosis in the field, to guide treatment. Despite their potential impact on the health of endangered species, knowledge of the epidemiology and pathophysiology of clostridial diseases in free-ranging wildlife remains limited (Silva & Lobato, 2015), and guidelines for management of disease are lacking.

The objectives of this review are to interrogate the scientific literature as well as historical cases in rhinoceroses and related species to formulate a clearer understanding of the epidemiology of clostridial diseases and use this information to make recommendations for management of disease (prevention, diagnosis, and control) in rhinoceroses. This will identify key knowledge gaps and provide guidelines for veterinarians and managers of free-ranging rhinoceros populations.

2 | HISTORICAL CASE REPORTS OF CLOSTRIDIAL DISEASE IN RHINOCEROS

The earliest reported case of clostridial disease in a rhinoceros dates to 1968, when an adult black rhinoceros succumbed to *P. sordellii* infection (Chafee, 1968). Since then, there has been an increase in disease reports in captive and free-ranging rhinoceros (Appendix S1). In the past two decades, numerous deaths have been reported, including 9 black rhinoceros (*Diceros bicornis*) that died from *C. perfringens* enterotoxaemia in Laikipia, Kenya; 28 white rhinoceros (*Ceratotherium simum*) that died from *C. novyi* and *P. sordellii* infections in North West province, South Africa; and 3 white rhinoceros affected by *C. perfringens* enterotoxaemia in a private conservation center in the USA (Citino et al., 2017; Ndeereh et al., 2012; Otto, 2014). A total of 31 morbidities and 43 mortalities were identified through the literature search and reports, with a higher proportion being reported in free ranging (30 morbidities and 39 mortalities) as opposed to captive rhinoceros. The most prevalent species were *C. perfringens*, *P. sordellii*, and *C. novyi*.

2.1 | Clostridium perfringens

2.1.1 | Epidemiology

C. perfringens enterotoxaemia was diagnosed as the cause of death in nine eastern black rhinoceroses on the Ol Jogi Conservancy (OJC) in Laikipia, Kenya over a 3-month period (May–July) in 2010 (Ndeereh et al., 2012). Climatic changes were observed in the period surrounding the outbreak; the long rains season (March–May 2009) had a 76.7% decrease in rainfall compared to the average of the preceding 5 years. This was followed in 2010 by intense rainfall that coincided with the outbreak, with an increase of 84% over the same period in 2009 (representative rainfall data from Mpala Research Centre). The increased rainfall after the dry period led to rapid growth of highly digestible vegetation (Meteer, 2017), which may have altered gut microbiota and precipitated the proliferation of *C. perfringens* (Geor, 2010; Lopes & Johnson, 2017).

Of the nine rhinoceros mortalities, 4 (44.4%) were adults (above 7 years of age), 2 (22.2%) were sub-adults (between 2 and 7 years of age) and 3 (33.3%) were calves (between 0 and 2 years of age). The age structure of the rhinoceros population at the time of the outbreak is unknown. Gender demographics were also not available; however, 5 female and 4 male rhinoceroses were affected (Ndeereh et al., 2012). At the time of the outbreak, 77.8% (7/9) of the rhinoceroses that died were unvaccinated. The other two animals were vaccinated after the first seven deaths, but died shortly after vaccination (one on the same day and the second approximately a month after), which suggests that there was insufficient time to develop immunity (Ndeereh et al., 2012).

C. perfringens has been diagnosed in cases of sudden death in captive settings; in three white rhinoceroses in a private conservation center in Florida (USA), and one case of illness in a zoo in the Czech Republic (Citino et al., 2017; Vahala et al., 1995). There are also anecdotal reports of cases of illness in other zoo rhinoceroses (M. Miller, pers. comm.).

2.1.2 | Clinical signs

All nine rhinoceroses at OJC displayed signs of colic, including grunting, rolling on the ground, dyspnea/tachypnoea, and diarrhea, and subsequently died within

2–5 h of exhibiting clinical signs (Ndeereh et al., 2012). In zoological settings, *C. perfringens* in rhinoceroses presented as diarrhea (Vahala et al., 1995) and as sudden death with no clinical signs (Citino et al., 2017). No further reports were identified describing clinical manifestations of *C. perfringens* in rhinoceroses.

2.1.3 | Treatment/intervention

Only one of the nine fatal cases at OJC received treatment, using flunixin meglumine (M. Mutinda, pers. comm.). Ten black rhinoceros identified to be at risk of developing disease were given prophylactic antibiotics (Duplocillin® LA, MSD Animal Health, Wellington New Zealand) consisting of 150,000 IU of benzathine penicillin and 150,000 IU of procaine penicillin per milliliter. The multivalent bacterin-toxoid ULTRABAC® 7 (Pfizer Animal Health, NY, USA) was also administered, which contains killed standardized cultures of *C. perfringens* types B, C, and D, *C. chauvoei*, *C. septicum*, *C. novyi* and *P. sordellii*. Treated rhinoceros were immobilized and subsequently administered 100 mL of the antibiotic intramuscularly (IM) and 5 mL of the vaccine subcutaneously (SC). In addition to these treatments, all 10 animals were relocated to an adjacent ranch where resident rhinoceros were unaffected. Eight of the 10 animals did not develop any signs of clostridial infection following translocation. The other two rhinoceroses (the same two mentioned above as vaccinated) died following translocation (Ndeereh et al., 2012).

Surviving animals at the Florida conservation center were vaccinated using *C. perfringens* type A and C (Novartis Animal Health, US, Inc., Larchwood, Iowa, 51241 USA), and type D toxoids (Professional Biological Company, Denver, Colorado, 80216 USA), and no further morbidities or mortalities were observed (Citino et al., 2017).

2.1.4 | Diagnostic testing and pathological findings

Due to the acute nature of clostridial disease, antemortem diagnostic tests were not performed on the free-ranging rhinoceroses. Diagnosis instead relied on gross necropsy findings and histopathology. All nine animals were in good body condition with scores of 3.5–4 (on a 1–5 scale). The most notable external finding on the carcasses was evidence of diarrhea as indicated by fecal staining of the perineum. The intestinal tract showed the most significant pathological changes, including congestion, edema, and hemorrhagic fluid contents (Ndeereh et al., 2012). The ileum, specifically near the ileo-cecal junction, appeared to be the most severely affected with extensive hemorrhage (M. Mutinda, pers. comm.).

Histopathological findings included necrotizing hemorrhagic enteritis with loss of intestinal epithelial detail, and lymphocyte and neutrophil infiltration in the intestinal walls (Ndeereh et al., 2012). *Clostridium* species appeared as Gram-positive rods along the walls of the intestines.

Intestinal contents from various parts of the gastrointestinal tract (GIT) and tissue samples from the brain, liver, and kidneys were also collected in cool boxes with ice packs for bacteriological culture (Ndeereh et al., 2012). *C. perfringens* was isolated from stomach contents through enriched culture media, but no toxin analysis was performed (Ndeereh et al., 2012).

2.2 | *Paeniclostridium sordellii* and *Clostridium novyi*

2.2.1 | Epidemiology

A rhinoceros captive breeding operation (CBO) in South Africa experienced 28 mortalities and over 30 morbidities caused by *P. sordellii* and *C. novyi* in their white rhinoceros population in 2014. The majority (89%) of cases were characterized by sudden death and occurred over 3 months (February to April) (Otto, 2014). Similar to the outbreak at OJC, a period of drought followed by heavy rain was reported prior to the outbreak of disease. The CBO property experienced 33%–34% below average rainfall in December 2013 and January 2014. During the disease outbreak, there was a dramatic increase in rainfall of 27% and 62% above average rainfall for February and March, respectively (Otto, 2014).

The majority (96.4%) of deaths occurred in adult animals with the remaining 3.6% deaths in sub-adults. Females comprised 85.7% of mortalities, whilst 14.3% were males. Most (79.2%) of the females that died were pregnant or with a calf. The age and sex demographics of the population at the time of the outbreak were not reported (Otto, 2014). Three other white rhinoceroses were confirmed to have died a year earlier from *C. novyi* infection in the CBO but no reports on this were identified.

2.2.2 | Clinical signs

Clinical signs were only observed in 10.7% (3/28) of rhinoceroses that died at the CBO, and included serous nasal discharge, bilateral lacrimation, abdominal

distension, stumbling and/or falling. Death occurred between 4 and 7 days post-onset of clinical signs despite three of these animals receiving treatment (Otto, 2014).

A black rhinoceros in a zoo that died of *P. sordellii* infection in 1968 exhibited abnormal behavior including weaving, panting, recumbency and open-mouth breathing and succumbed 3 days after the onset of clinical signs (Silberman & Fulton, 1979).

2.2.3 | Treatment/intervention

After three mortalities due to *C. novyi* were confirmed in February 2013 in a Northern property on the same CBO, prophylactic vaccination was initiated using the multi-clostridial inactivated Covexin® 10 vaccine (MSD Animal Health, South Africa). This vaccine covers infection by *C. perfringens* types A, B, C, and D, *C. chauvoei*, *C. novyi* type B, *C. septicum*, *C. tetani*, *P. sordellii*, and *C. haemolyticum* (*C. novyi* type D) (Otto, 2014). Unfortunately, the disease outbreak began on a southern property of the CBO where individuals were unvaccinated. Of 28 mortalities that occurred across the whole CBO, 50% (14/28) were unvaccinated, 17.9% (5/28) had received only one vaccination, and 32.1% (9/28) had received two vaccinations following the specific recommended vaccine administration regime of two doses four to 6 weeks apart. Among the rhinoceroses that were fully vaccinated, 64% (9/14) became ill and subsequently died during the outbreak. Of these, 22% (2/9) died 1 week after receiving the second vaccination, 33% (3/9) died 2 weeks after receiving the second, and 44% (4/9) died 3 weeks after receiving the second vaccination (Otto, 2014).

More than 30 other rhinoceroses were observed to exhibit clinical signs and were successfully treated with unspecified long-acting penicillin, multi-vitamin injection, and an anti-inflammatory toxoid binder. Varying responses were recorded to this treatment, but 3 rhinoceroses still died four to 7 days after treatment was administered (Otto, 2014).

2.2.4 | Postmortem diagnostic testing and findings

Postmortem examination was carried out on 22 of the 28 rhinoceroses while the remaining were in a state of advanced decomposition. On external examination, the rhinoceroses presented with bleeding from the mouth, nose, ears, rectum, and skin. Carcasses were severely bloated despite necropsy procedures being conducted within 12 h (Otto, 2014). A set of tissues were collected in formalin from rhinoceroses that died at the CBO, as well as blood smears and impression smears from the liver, lung, heart and small intestines for cytology and direct fluorescent antibody testing (FAT) (Otto, 2014). Gross necropsy findings in all 22 carcasses revealed congestion, edema, and hemorrhage of both small and large intestines, hemorrhagic contents of the small intestines, and bloated ascending colon and caecum. Segmental pathology of the intestines was observed in the jejunum (all carcasses), ascending duodenum (18/22) and the ileum (16/22) (Otto, 2014).

Gross and histopathological changes were consistent with necrotizing hemorrhagic enteritis, with hemorrhagic shock being the cause of death in the rhinoceros. On histopathology, the loss of intestinal mucosal detail and villi tips was observed in 50% of the rhinoceroses, while pulmonary congestion (60.7%) and emphysema (39.3%) and hepatic autolysis (39.3%) were also common findings. The histopathological findings are summarized (Appendix S2). *Clostridium* species were identified in blood and impression smears from 8 of the rhinoceroses, where histopathological analysis identified numerous rod-shaped bacteria with budding ends and central spores. Direct FAT results were often negative with only four samples having positive results (Otto, 2014).

3 | DISCUSSION

Clostridial diseases are an under-recognized threat to the conservation of rhinoceroses. Three aspects associated with the case reports that we reviewed cause particular concern for rhinoceros' population health: (1) disease has been observed to occur in clusters with high morbidity and mortality within very brief periods, anecdotally linked to weather extremes; (2) both black and white rhinoceros were affected; (3) the acute nature of the diseases limit prompt veterinary intervention (Ndeereh et al., 2012; Otto, 2014). Though only Kenya and South Africa have reported clostridial cases in non-captive rhinoceros, it is likely that outbreaks have gone undetected in other range countries where there are differences in wildlife disease surveillance capacity.

3.1 | Preventative measures

Studies of clostridial diseases in domestic animals have identified that predisposing factors affecting pathogenesis differ across host species. Sudden changes in feed, environmental conditions promoting rapid growth of vegetation, and the presence of other physiological stressors are considered risk factors that can result in the rapid multiplication of bacteria and toxin production, and

TABLE 1 Predisposing factors of clostridial disease in rhinoceroses.

Predisposing factor	Reference(s)
Exogenous factors	
Climatic variation: extremes associated with climate change and normal patterns of drought—and the resulting sudden dietary changes.	Geor (2010) Lopes and Johnson (2017) Meteer (2017) Ndeereh et al. (2012) Otto (2014)
Stress—capture and immobilization	Dickens et al. (2010) Sainsbury and Vaughan-Higgins (2012) Teixeira et al. (2007)
Land management activities, for example, excavations for dams	Otto (2014)
Population exceeding ecological carrying capacity	Emslie et al. (2009)
Host factors[a]	
Adults	Chafee (1968) Citino et al. (2017) Ndeereh et al. (2012) Otto (2014)
Females	Citino et al. (2017) Ndeereh et al. (2012) Otto (2014)
Pregnancy status—in and with calf	Citino et al. (2017) Ndeereh et al. (2012) Otto (2014)
Vaccination status	Ndeereh et al. (2012) Otto (2014)

[a]Putative risk factors as the respective age and sex demographics, as well as the pregnancy and vaccination status of the entire rhinoceros populations at the time of the outbreaks are unknown.

subsequently disease (Hing et al., 2016). It is therefore important that rhinoceros health managers are vigilant of predisposing factors to clostridial disease so that preventative measures can be instituted prior to outbreaks occurring. Table 1 summarizes the identified predisposing factors.

3.1.1 | Signalment

The putative individual animal risk factors identified in this review include age, sex, and pregnancy status.

Of the cases that we reviewed, 79.5% of clostridial disease mortalities occurred in adults. In captive rhinoceros, elephants, and horses, adults are also most commonly affected (Bacciarini et al., 2001; Citino et al., 2017; Costa et al., 2022; Das et al., 2008; Traub-Dargatz & Jones, 1993). This may be explained by the different feeding habits as dictated by the physical, physiological, and microbiome developmental changes that occur within the gastrointestinal tract with age (Lawrence & Lawrence, 2009).

Though no sex predisposition for clostridial infection has been identified in reports, the review observed a skew towards females in rhinoceroses. In captivity, rhinoceros and elephants diagnosed with enteric clostridial disease have all been female, although it is important to note that zoological collections are often heavily skewed towards females (Bacciarini et al., 2001; Costa et al., 2022; Das et al., 2008).

Among the affected females 15.6% (5/32) were in calf ranging from three to 16 months pregnant, and 53.1% (17/32) were with calves. Pregnancy can cause maternal immunological changes, with large increases in progesterone and estrogen having been identified as causing immunosuppression (Abu-Raya et al., 2020; Kourtis et al., 2014; Pazos et al., 2012). This could make pregnant females more vulnerable to clostridial infection. The physiological stress of nursing and protecting offspring, could explain why a higher proportion of affected females had calves (Clinchy et al., 2013). However, being a CPO, the population is also skewed towards pregnant females and those with calves.

3.1.2 | Rhinoceros medical and vaccination history

Clostridial disease may be associated with (1) previous illness/injury that lowered immunity giving the opportunity for secondary clostridial infection; and (2) stressful events involving capture and immobilization for treatment, as stress is known to be immunosuppressive (Dickens et al., 2010; Sainsbury & Vaughan-Higgins, 2012; Teixeira et al., 2007). Obtaining an accurate medical history—including vaccination status—is therefore crucial.

3.1.3 | Environmental conditions

Clostridial disease outbreaks in domestic and wild animals have often been associated with significant dry periods preceding intensive rainfall (de Vos, 1980; Lebrun et al., 2010; Ndeereh et al., 2012; Otto, 2014). Such conditions could favor sporulation of dormant clostridial spores in the environment, which are then ingested by rhinoceroses. The abrupt heavy rainfall also leads to rapid increases in the digestibility of vegetation, causing

changes within the microbiome and triggering proliferation of *Clostridium* (Lewis, 2011; Ndeereh et al., 2012).

Certain land management activities and the ecological carrying capacity (ECC) of rhinoceros within conservation areas are also potential predisposing factor to clostridial disease. Deep excavations and creation of earth-filled dams could lead to increased sporulation of *Clostridium* in soils and were reported in the CBO (Otto, 2014). More densely populated rhino conservation areas could face larger outbreaks as individuals congregate around available resources and are therefore more likely to be exposed to the predisposing factors such as lush vegetation (Emslie et al., 2009). Clostridial diseases may be of greater conservation importance in free-ranging rhinoceroses than captive populations. Captive facilities offer more intense health and husbandry practices, which may prevent or at least mitigate the serious effects of disease (Bais et al., 2017). As free-ranging rhinoceroses are not sheltered, they are expected to have higher exposure and vulnerability to environmental elements and anthropogenic changes which are predisposing factors to development of clostridial disease. Furthermore, wildlife veterinary capacity across Africa is generally limited, bringing into question the true prevalence of the disease through undiagnosed or unreported cases.

3.2 | Disease presentation

The most common clinical presentation in free-ranging rhinoceroses with clostridial disease is sudden death. Even in captive wildlife settings where animals are monitored more closely, rhinoceros and elephants have died within 2–5 h of onset of clinical signs (Bacciarini et al., 2001; Citino et al., 2017; Costa et al., 2022; Das et al., 2008).

Since the rapid multiplication and toxin production of *C. perfringens* within the GIT of animals results in enterotoxaemia, clinical signs are primarily indicative of GIT disturbance. Captive Asian and African elephants diagnosed with *C. perfringens* also present with acute lethargy, colic, recumbency and diarrhea (Bacciarini et al., 2001; Costa et al., 2022; Das et al., 2008). Infections with *P. sordellii* also progress rapidly due to toxic/septicemic shock. In horses, death occurs within 24–36 h of onset of clinical signs, which include acute colic, lethargy, and depression (Nyaoke et al., 2020). *C. novyi* similarly causes per acute to acute fatal disease with mortalities in horses having been observed within 12–72 h. Clinical signs may be absent but colic, ataxia, depression, and recumbency may sometimes be seen (Aiello et al., 2016; Nyaoke et al., 2018; Uzal et al., 2022).

3.3 | Disease management

3.3.1 | Treatment

There is limited knowledge on the most effective prevention and treatment protocols for clostridial disease in rhinoceroses and wildlife more broadly (Nyaoke et al., 2020; Otter & Uzal, 2020; Songer, 1996; Traub-Dargatz & Jones, 1993; Uzal et al., 2022). Treatment is often unrewarding due to per acute disease (Jerram, 2019; Traub-Dargatz & Jones, 1993). Treatment of captive elephants with various antibiotic treatments, including tetracyclines and sulphonamides has been unsuccessful (Bacciarini et al., 2001; Das et al., 2008). Penicillin derivatives, followed by ampicillin, clindamycin, and metronidazole have been identified as the antibiotics of choice but caution must be taken in administration of metronidazole in pregnant rhinoceros due to its suspected teratogenic properties (Costa et al., 2022; Das et al., 2008; Plumb, 2018; Vidor et al., 2015). Careful antibiotic selection is important since drug-resistant strains of *Clostridium* spp. have been identified (Abel-Santos, 2015; Rood & Cole, 1991; Vidor et al., 2015).

In addition to specific antimicrobial therapy, supportive treatment is vital in sustaining the physiological well-being of affected rhinoceros. This includes the administration of isotonic intravenous fluids or rectal enemas for treatment of toxemia and dehydration, and nonsteroidal anti-inflammatory drugs (NSAIDs) such as flunixin meglumine and phenylbutazone. NSAIDs should only be given to hydrated rhinoceros to avoid renal damage (MacAllister et al., 1993; Plumb, 2018; Traub-Dargatz & Jones, 1993). Affected rhinoceros could also benefit from multivitamin injections, which promote regeneration of damaged intestinal mucosal epithelial lining and can contribute to balance of the GIT microbiota (Pham et al., 2021).

Treatment of *C. novyi* is often unrewarding and therefore vaccination is the only effective management option (Uzal et al., 2022).

3.3.2 | Vaccination

Vaccines recommended for clostridial disease management in wildlife are generally used off-label (Springer & Selbitz, 1999). Jerram (2019) and Khiav and Zahmatkesh (2021) describe the commercial clostridial vaccines administered to domestic animals and analyze their varying efficacies, as well as advantages and disadvantages. The different vaccines used in rhinoceroses identified in this review, the *Clostridium* species for which they offer immunization, and the recommended administration

regimen by the manufacturers are summarized (Appendix S3).

Rhinovax® has been formulated for immunization of wildlife species against *C. perfringens* type A, *C. chauvoei*, *C. novyi* types A and B, *C. septicum*, and *P. sordellii*, as well as anthrax (*Bacillus anthracis*) (Balfour et al., 2019). The vaccine was developed for use in white rhinoceros, African buffalo, sable antelope, roan antelope, nyala, and lions, and has demonstrated effectiveness in laboratory and field trials in Namibia (Tubbesing, 2017). Since clostridial disease outbreaks may be linked to heavy rainfall, all vaccinations should be completed before the beginning of the rainy seasons with a recommended booster vaccination 3–4 weeks after the initial vaccine dose. This should be followed by annual boosters, particularly in regions identified to be prone to clostridial outbreaks. As stress may also increase susceptibility to infection (Agarwal & Marshall Jr, 2001), initial vaccine doses should be administered 2–3 weeks prior to any planned immobilizations and translocations, and when wildlife are confined to a boma (Hing et al., 2016). Since it takes up to 2 weeks for effective immunity to develop, any exposure to clostridial pathogens or toxins within this period may still result in disease. Localized reactions at the site of vaccinations have been reported, but no other adverse effects have been observed (Citino et al., 2017). While immune responses to clostridial vaccination have been tested in captive and semi-captive rhinoceros, variations in serological responses call for more thorough evaluation of differing vaccine effectiveness in rhinoceros species (Buys et al., 2020; Citino et al., 2017).

From the vaccinated individuals, 8 rhinos died between 2 and 4 weeks after receiving their vaccination which is after the period in which the vaccines should have conferred immunity. This raises questions on the true efficacy of the vaccine types used, vaccine delivery, or if other factors inherent to the individual hosts were at play resulting in a failed immune response, such as the presence of maternal antibodies in calves or stress precipitated by capture. These varied immune responses to vaccination warrant further studies to reveal more effective vaccine types and regimen for clostridial disease in rhinoceroses, identifying which specific host factors may inhibit development of immunity.

3.3.3 | Translocations

Translocations have been used as a preventative measure for at-risk rhinoceros, to remove the individuals from suspected predisposing factors. However, the costs of such an intervention—both monetary and in terms of the physical and physiological stress associated with capture and movement of rhinoceros (which may also increase the risk of enteric clostridial disease development)—should be carefully evaluated (Dickens et al., 2010; Sainsbury & Vaughan-Higgins, 2012; Teixeira et al., 2007).

3.4 | Diagnostic testing

Obtaining a definitive diagnosis first relies on proper history taking of the disease occurrence, including collecting data about the affected individual rhinoceros, location, and environment. Knowledge on the location of clostridial disease outbreak is vital as it could be an area known to have frequent outbreaks and past disease management efforts can inform interventions.

For ante-mortem diagnosis, fresh samples should be collected from the center of multiple fecal boluses and submitted for anaerobic culture and toxin detection. These should be collected in sterile containers and refrigerated or placed on ice packs immediately as toxins are known to degrade rapidly, with some toxins being destroyed within 4–6 h of death. If delivery to the laboratory will be delayed, samples should be frozen at −20°C and transported frozen to laboratories for anaerobic culture and toxin detection (Traub-Dargatz & Jones, 1993).

3.4.1 | Necropsy and histopathology

Necropsy must be performed as soon as possible after death. The exposure of rhinoceros carcasses to environmental elements such as elevated temperatures, increase the rate of carcass decomposition. Commensal *Clostridium* spp. multiply rapidly within the GIT after death, complicating diagnosis and leading to misleading pathological findings (Otter & Uzal, 2020).

Necropsies should be carried out systematically and following a comprehensive checklist to avoid missing any valuable information. General observation of the rhinoceros' immediate environment could be indicative of colic signs such as rolling on the ground and recumbency (Otto, 2014). Segmental hemorrhagic and necrotizing enteritis is consistently observed across species with enteric clostridial infection, including rhinoceros, elephants, horses, pigs, lorikeets, and cattle (Costa et al., 2022; Das et al., 2008; Diab et al., 2012; Grau-Roma et al., 2021; Lebrun et al., 2010; Ndeereh et al., 2012; Posthaus et al., 2020). The systemic circulation of toxins results in pathological changes (e.g., congestion and edema) of other tissues and organs including the lungs, heart, and mesenteric lymph nodes (Das et al., 2008; Ndeereh et al., 2012; Otto, 2014). Carcasses of *C. novyi* infections decompose rapidly with common gross findings including congested

TABLE 2 Summary of recommended samples to be collected and diagnostic tests.

Specimen	Preservation	Diagnostic test
Ante-mortem		
Feces—25 g (20–30 mL if liquid feces)	Refrigerate[a]	Aerobic and anaerobic bacterial culture
	Freeze[b]	Assay for enterotoxins
Rectal swab	Refrigerate[a]	Aerobic and anaerobic bacterial culture
Post-mortem		
Intestinal content[c] (20–30 mL)	Freeze[b]	Assay for enterotoxins
Generous section of intestine (5–10 cm of both severely affected and marginal sections tied off to contain contents)	Refrigerate[a]	Anaerobic bacterial culture
Representative sections of intestine, that is, duodenum to colon (2–3 cm in length each)	10% buffered formalin	Histologic examination
Impression smear of affected intestinal mucosa, intestinal content, lung, heart.	Air dry	Gram stain
Other parenchymal organs—liver, kidney, spleen, lung, heart.	10% buffered formalin	Histologic examination

Note: If no methods of preserving samples are available, samples should arrive at laboratory within 1 to 2 h from collection. Adapted from Traub-Dargatz and Jones (1993) and Otter and Uzal (2020).
[a]Refrigeration for 48–72 h will adequately preserve samples for culture.
[b]Samples for toxin analysis should be frozen quickly after collection.
[c]The terminal small intestine (ileum) is the preferred site for intestinal content collection.

subcutaneous blood vessels, hepatomegaly with necrotic foci, endocardial hemorrhage, and accumulation of excess fluids within the pericardial sac and thoracic and abdominal cavities (Underwood et al., 2015; Uzal et al., 2022).

Appropriate collection, preservation, and transport of clinical samples for laboratory-based confirmatory tests are vital. The vast size and remoteness of conservation areas and poor infrastructure limit disease investigations in free-ranging wildlife. As a result, laboratories are often inaccessible, necessitating adjustments in sample preservation to include storage of samples in cool boxes with ice packs for samples requiring refrigeration and use of portable liquid nitrogen tanks for samples that need to be frozen. Table 2 summarizes the recommended samples, their preservation and diagnostic tests.

Histopathological findings are in line with the gross post-mortem findings indicating a necrotizing hemorrhagic enteritis. *Clostridium* species appearing as Gram-positive rods either within the lumen or along the walls of the intestines (Diab et al., 2012; Ndeereh et al., 2012).

3.4.2 | Bacteriology

Culture is important for isolation and identification of *Clostridium* species but it is also vital for carrying out antimicrobial susceptibility testing to identify the most effective antibiotics for management of disease (Costa et al., 2022; Das et al., 2008). As culture and sensitivity testing can take up to 5 days, findings may not support treatment of acute disease but are vital in informing prophylaxis for other at-risk rhinoceros as well as prompt treatment of those that develop similar clinical signs in the future. Bacteriology tests have been described for both *C. perfringens* and *P. sordellii* (De La Fe et al., 2006; Nyaoke et al., 2020; Uzal & Songer, 2008).

C. novyi is a strict anaerobe, extremely sensitive to exposure to oxygen. This, and its fastidious nutritional growth requirements, makes it difficult to culture and isolate, requiring molecular and serological tests for definitive diagnosis (Oaks et al., 1997; Uzal et al., 2022).

3.4.3 | Toxin detection and analysis

Detection and identification of clostridial toxins is vital in attaining a confirmatory diagnosis (Traub-Dargatz & Jones, 1993; Uzal et al., 2022). *C. perfringens* is classified into seven biotypes (A–G) based on the combination of toxins produced (Rood et al., 2018). *C. perfringens* type A is not considered an important enteric pathogen in horses as this toxin type has been isolated from feces of clinically healthy horses and other domestic animals, raising questions on its role in disease development (Songer, 1996; Uzal et al., 2010, 2016). Isolation of the other types is of pathological significance. *P. sordellii* produces two major toxins known as the lethal toxin and hemorrhagic toxin. The lethal toxin (TcsL) is implicated in clostridial enteritis (Abel-Santos, 2015). *C. novyi* is primarily a soilborne pathogen. *C. novyi* type B has been identified within the intestines and liver of healthy domestic animals, but information is lacking on this in rhinoceroses and other wildlife species. There are 4 types (A–D) based on the

toxins produced (Oaks et al., 1997; Stämpfli & Oliver-Espinosa, 2021).

Three methods have been described for detection of toxins in the literature; (1) polymerase chain reaction (PCR) on frozen liver samples for *C. novyi*, and intestinal sections for *C. perfringens* and *P. sordellii* (Albini et al., 2008; Costa et al., 2022; Diab et al., 2012; Schoster et al., 2012; Uzal et al., 2022); (2) enzyme-linked immunosorbent assay (ELISA) on contents of the small and large intestines (Diab et al., 2012; Nyaoke et al., 2020); and (3) immunohistochemistry on formalin-fixed paraffin-embedded sections, 4–5 μm in thickness, of the liver, ileum, and colon (Bacciarini et al., 2001; Diab et al., 2012; Nyaoke et al., 2020; Oaks et al., 1997; Otter & Uzal, 2020).

4 | CONCLUSION

This review identified knowledge gaps, particularly on (1) host risk factors, (2) anthropogenic risk factors and, (3) effective disease management options on clostridial diseases in rhinoceroses, as well as other wildlife species. Adequate knowledge on these will help direct prevention efforts appropriately. This prompts further research questions including:

- Which age and sex of rhinoceroses are at a higher risk of developing clostridial disease infection?
- Does the pregnancy status of female rhinoceroses adequately alter immunity to make pregnant individuals have a higher predisposition to clostridial disease development?
- Do rhinoceros conservation efforts involving immobilization and translocation of rhinoceros for protection and population health management serve as predisposing factors in the development of enteric clostridial disease?
- How do climate-mediated vegetation nutrient fluxes affect rhinoceroses gut microbiome and lead to clostridial disease development?
- Which is the most effective vaccine and administration regimes to be employed across free-ranging rhinoceros populations?

Clostridial diseases pose a threat to rhinoceros populations and should be included in the differential diagnosis for cases of acute colic and sudden death, and where segmental hemorrhagic enteritis is observed on necropsy. Environmental conditions of severe drought and intense rainfall are putative risk factors. The identification and isolation of *Clostridium* species alone does not confirm the role of the bacteria in disease. Toxin detection and identification must be carried out for a confirmatory diagnosis.

The increase in drought risk expected across Africa will support the survivability of resistant clostridial spores in the environment, while heavy rainfall events will favor their sporulation and the rapid growth of vegetation predisposing rhinoceroses to disease (Ahmadalipour et al., 2019; Haile et al., 2020). The inevitability of anthropogenic effects to rhinoceros' habitats and climate change highlight the importance of addressing the challenges brought by clostridial diseases in these high value species (Emslie et al., 2009; Gallana et al., 2013).

It will be vital to gain an in-depth understanding of clostridial disease dynamics in relation to anthropogenic stressors including conservation management activities involving rhinoceros immobilization and translocation. These activities subject individual animals to both physical and physiological stress, changes in diet, and increased stocking densities (Buys et al., 2020; Cunningham, 1996; Hing et al., 2016; Woodford & Rossiter, 1994). Given the need to extend rhinoceros habitat ranges and still increase their security, there is a requirement to better equip rhinoceros stakeholders to make conservation decisions such as selection criteria for both individual rhinoceros and locations for translocation.

AUTHOR CONTRIBUTIONS

Mathew Mutinda and Suzan Murray conceived of and designed the research. Mathew Mutinda and Shaleen Angwenyi acquired the data. Shaleen Angwenyi, James Hassell and Michele Miller analyzed and interpreted the data. Shaleen Angwenyi drafted the manuscript. All authors contributed to critically revising the manuscript, gave final approval of the version to be published, and agree to be accountable for all aspects of the work.

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CONFLICT OF INTEREST STATEMENT

The authors declare no potential conflict of interest.

DATA AVAILABILITY STATEMENT

The authors confirm that the data supporting the findings of this study are available within the article [and/or] its supplementary materials.

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REFERENCES

Abel-Santos, E. (2015). Endospores, sporulation and germination. In *Molecular Medical Microbiology* (Vol. 1, pp. 163–178). Academic Press. https://doi.org/10.1016/B978-0-12-397169-2.00009-3

Abu-Raya, B., Michalski, C., Sadarangani, M., & Lavoie, P. M. (2020). Maternal immunological adaptation during normal pregnancy. *Frontiers in Immunology*, *11*, 575197. https://doi.org/10.3389/fimmu.2020.575197

Agarwal, S. K., & Marshall, G. D., Jr. (2001). Stress effects on immunity and its application to clinical immunology. *Clinical & Experimental Allergy*, *31*(1), 25–31.

Ahmadalipour, A., Moradkhani, H., Castelletti, A., & Magliocca, N. (2019). Future drought risk in Africa: Integrating vulnerability, climate change, and population growth. *Science of the Total Environment*, *662*, 672–686. https://doi.org/10.1016/j.scitotenv.2019.01.278

Aiello, S. E., Moses, M. A., & Allen, D. G. (2016). *The Merck veterinary manual* (p. 3325). Merck & Company, Incorporated.

Albini, S., Brodard, I., Jaussi, A., Wollschläger, N., Frey, J., Miserez, R., & Abril, C. (2008). Real-time multiplex PCR assays for reliable detection of *Clostridium perfringens* toxin genes in animal isolates. *Veterinary Microbiology*, *127*(1–2), 179–185. https://doi.org/10.1016/j.vetmic.2007.07.024

Bacciarini, L. N., Gröne, A., Pagan, O., & Frey, J. (2001). *Clostridium perfringens* β2-toxin in an African elephant (*Loxodonta africana*) with ulcerative enteritis. *Veterinary Record*, *149*(20), 618–620. https://doi.org/10.1136/vr.149.20.618

Bais, B., Tak, L., & Mahla, S. (2017). Study of preventive health measures for wildlife in captivity: A review of management approaches. *Int J Avian Wildl Biol*, *2*(3), 73–75.

Balfour, D., Shaw, J., Banasiak, N., le Roex, N., Rusch, U., & Emslie, R. (2019). *Concise best practice guidelines for the biological management of African rhino* (p. 123). WWF-SA.

Buys, A., Crafford, J., & Van Heerden, H. (2020). Development and evaluation of indirect enzyme-linked immunosorbent assays for the determination of immune response to multiple clostridial antigens in vaccinated captive bred southern white rhinoceros (*Ceratotherium simum simum*). *Acta Veterinaria Scandinavica*, *62*(1), 1–8.

Chafee, P. S. (1968). Report on the death of a rhinoceros. *Journal of Small Animal Practice*, *9*, 133–134.

Citino, S. B., Goe, A., Metrione, L., Oliva, M., & Garner, M. (2017). Sudden death in three southern white rhinoceros (*Ceratotherium simum simum*) secondary to presumptive *Clostridium perfringens* enterotoxaemia. *Proceedings of the American Association of Zoo Veterinarians*, *2017*, 105–106.

Clinchy, M., Sheriff, M. J., & Zanette, L. Y. (2013). Predator-induced stress and the ecology of fear. *Functional Ecology*, *27*(1), 56–65.

Costa, T., Rocchigiani, G., Zendri, F., Drake, G., Lopez, J., Chantrey, J., & Ricci, E. (2022). Elephant endotheliotropic herpesvirus 4 and *Clostridium perfringens* type C fatal co-infection in an adult Asian elephant (*Elephas maximus*). *Animals*, *12*(3), 349.

Cunningham, A. A. (1996). Disease risks of wildlife translocations. *Conservation Biology*, *10*(2), 349–353. https://doi.org/10.1046/j.1523-1739.1996.10020349.x

Das, A., Mazumder, Y., Dutta, B. K., Shome, B. R., Bujarbaruah, K. M., & Sharma, G. D. (2008). *Clostridium perfringens* type A beta2 toxin in elephant (*Elephas maximus indicus*) and pygmy hog (*Sus salvanius*) with haemorrhagic enteritis in Assam, India. *African Journal of Microbiology Research*, *2*(8), 196–201.

De La Fe, C., Rodríguez, J. M., Ramírez, G. A., Hervás, J., Gil, J., & Poveda., J. B. (2006). Sudden death associated with *Clostridium sordellii* in captive lions (*Panthera leo*). *Veterinary Pathology*, *43*(3), 370–374. 10.1354%2Fvp.43-3-370

de Vos, V. (1980). Black rhino *Diceros bicornis* minor mortality in the Kruger National Park. *Koedoe*, *23*(1), 188–189. https://doi.org/10.4102/koedoe.v23i1.647

Diab, S. S., Kinde, H., Moore, J., Shahriar, M. F., Odani, J., Anthenill, L., Songer, G., & Uzal, F. A. (2012). Pathology of *Clostridium perfringens* type C enterotoxemia in horses. *Veterinary Pathology*, *49*(2), 255–263. 10.1177%2F0300985811404710

Dickens, M. J., Delehanty, D. J., & Romero, L. M. (2010). Stress: An inevitable component of animal translocation. *Biological Conservation*, *143*(6), 1329–1341. https://doi.org/10.1016/j.biocon.2010.02.032

Dublin, H., & Wilson, A. (1998). *The fight for survival: Four decades of conserving Africa's rhinos*. WWF International.

Dwyer, R., Goosen, W., Buss, P., Kedward, S., Manamela, T., Hausler, G., Chileshe, J., Rossouw, L., Fowler, J., Miller, M., & Witte, C. (2022). Epidemiology of Mycobacterium bovis infection in free-ranging rhinoceros in Kruger National Park, South Africa. *Proceedings of the National Academy of Sciences*, *119*(24), e2120656119.

Emslie, R., Amin, R., & Kock, R. (Eds.). (2009). *Guidelines for the in-situ re-introduction and translocation of African and Asian rhinoceros*. IUCN.

Emslie, R., & Brooks, M. (Eds.). (1999). *African rhino: Status survey and conservation action plan*. IUCN/SSC African Rhino Specialist Group. IUCN.

Gallana, M., Ryser-Degiorgis, M. P., Wahli, T., & Segner, H. (2013). Climate change and infectious diseases of wildlife: Altered interactions between pathogens, vectors and hosts. *Current Zoology*, *59*(3), 427–437. https://doi.org/10.1093/czoolo/59.3.427

Geor, R. J. (2010). Digestive strategy and flexibility in horses with reference to dietary carbohydrates. In *The impact of nutrition on the health and welfare of horses* (pp. 17–28). Wageningen Academic Publishers.

Grau-Roma, L., Navarro, M., Blatter, S., Wenker, C., Kittl, S., Uzal, F. A., & Posthaus, H. (2021). *Clostridium perfringens*–associated necrotic enteritis-like disease in coconut lorikeets (*Trichoglossus haematodus*). *Veterinary Pathology*, *58*(2), 423–427. 10.1177%2F0300985820971788

Haile, G. G., Tang, Q., Hosseini-Moghari, S. M., Liu, X., Gebremicael, T. G., Leng, G., Kebede, A., Xu, X., & Yun, X. (2020). Projected impacts of climate change on drought patterns over East Africa. *Earth's Futures*, *8*(7), e2020EF001502. https://doi.org/10.1029/2020EF001502

Hing, S., Narayan, E. J., Thompson, R. A., & Godfrey, S. S. (2016). The relationship between physiological stress and wildlife disease: Consequences for health and conservation. *Wildlife Research*, *43*(1), 51–60. https://doi.org/10.1071/WR15183

Jerram, L. (2019). Clostridial disease in cattle. *Livestock*, *24*(6), 274–279. https://doi.org/10.12968/live.2019.24.6.274

Jonyo, J. F. (1989). Doctoring rhinos: Diseases seen in Kenya. *Pachyderm*, *12*, 23–24.

Khan, M. K., & van Strien, N. J. (1997). *Asian rhinos: Status survey and conservation action plan*. IUCN.

Khiav, L. A., & Zahmatkesh, A. (2021). Vaccination against pathogenic clostridia in animals: A review. *Tropical Animal Health and Production*, *53*, 284. https://doi.org/10.1007/s11250-021-02728-w

Kourtis, A. P., Read, J. S., & Jamieson, D. J. (2014). Pregnancy and infection. *New England Journal of Medicine*, *370*(23), 2211–2218. https://doi.org/10.1056/NEJMra1213566

Lawrence, L. A., & Lawrence, T. J. (2009). Development of the equine gastrointestinal tract. *Advances in Equine Nutrition IV*, 173–183.

Lebrun, M., Mainil, J. G., & Linden, A. (2010). Cattle enterotoxaemia and *Clostridium perfringens*: Description, diagnosis and prophylaxis. *Veterinary Record*, *167*(1), 13–22. https://doi.org/10.1136/vr.167.1.12

Lewis, C. J. (2007). Clostridial diseases. *Diseases of Sheep*, 156–167.

Lewis, C. J. (2011). Control of important clostridial diseases of sheep. *Veterinary Clinics: Food Animal Practice*, *27*(1), 121–126. https://doi.org/10.1016/j.cvfa.2010.10.009

Lopes, M. A., & Johnson, P. J. (2017). Effects of feeding on equine gastrointestinal function or physiology. In *The equine acute abdomen* (pp. 66–77). John Wiley & Sons.

MacAllister, C. G., Morgan, S. J., Borne, A. T., & Pollet, R. A. (1993). Comparison of adverse effects of phenylbutazone, flunixin meglumine, and ketoprofen in horses. *Journal of the American Veterinary Medical Association*, *202*(1), 71–77.

Meteer, W. T. (2017). *Mitigating the challenges of grazing lush*. Spring Forages.

Miller, M. A., Buss, P., Parsons, S. D., Roos, E., Chileshe, J., Goosen, W. J., van Schalkwyk, L., de Klerk-Lorist, L. M., Hofmeyr, M., Hausler, G., Rossouw, L., Manamela, T., Mitchell, E., Warren, R., & van Helden, P. (2018). Conservation of white rhinoceroses threatened by bovine tuberculosis, South Africa, 2016–2017. *Emerging Infectious Diseases*, *24*(12), 2373–2375.

Ndeereh, D., Ouma, B. O., Gaymer, J., Mutinda, M., & Gakuya, F. (2012). Unusual mortalities of the eastern black rhinoceros (*Diceros bicornis michaeli*) due to clostridial enterotoxaemia in Ol Jogi pyramid sanctuary, Kenya. *Pachyderm*, *51*, 45–51.

Nyaoke, A. C., Navarro, M. A., Beingesser, J., & Uzal, F. A. (2018). Infectious necrotic hepatitis caused by *Clostridium novyi* type B in a horse: Case report and review of the literature. *Journal of Veterinary Diagnostic Investigation*, *30*(2), 294–299.

Nyaoke, A. C., Navarro, M. A., Fresneda, K., Diab, S. S., Moore, J., Lyras, D., Awad, M., & Uzal, F. A. (2020). Paeniclostridium (*Clostridium*) sordellii–associated enterocolitis in 7 horses. *Journal of Veterinary Diagnostic Investigation*, *32*(2), 239–245.

Oaks, J. L., Kanaly, S. T., Fisher, T. J., & Besser, T. E. (1997). Apparent *Clostridium haemolyticum*/*Clostridium novyi* infection and exotoxemia in two horses. *Journal of Veterinary Diagnostic Investigation*, *9*(3), 324–325.

Otter, A., & Uzal, F. A. (2020). Clostridial diseases in farm animals: 1. Enterotoxaemias and other alimentary tract infections. *In Practice*, *42*(4), 219–232. https://doi.org/10.1136/inp.m1462

Otto, M. (2014). *Field report on "sudden death" in white rhinoceros (*Ceratotherium simum*)*.

Pazos, M., Sperling, R. S., Moran, T. M., & Kraus, T. A. (2012). The influence of pregnancy on systemic immunity. *Immunologic Research*, *54*(1), 254–261. https://doi.org/10.1007/s12026-012-8303-9

Pham, V. T., Dold, S., Rehman, A., Bird, J. K., & Steinert, R. E. (2021). Vitamins, the gut microbiome and gastrointestinal health in humans. *Nutrition Research*, *95*, 35–53. https://doi.org/10.1016/j.nutres.2021.09.001

Plumb, D. C. (2018). *Plumb's Veterinary Drug Handbook: Desk*. John Wiley & Sons.

Posthaus, H., Kittl, S., Tarek, B., & Bruggisser, J. (2020). *Clostridium perfringens* type C necrotic enteritis in pigs: Diagnosis, pathogenesis, and prevention. *Journal of Veterinary Diagnostic Investigation*, *32*(2), 203–212. https://doi.org/10.1177/1040638719900180

Ramsay, E. C., & Zainuddin, Z. Z. (1993). Infectious diseases of the rhinoceros and tapir. *Zoo and Wild Animal Medicine*, *3*, 459–466.

Rood, J. I., Adams, V., Lacey, J., Lyras, D., McClane, B. A., Melville, S. B., Moore, R. J., Popoff, M., Sarker, M. R., Songer, J. G., Uzal, F. A., & Van Immerseel, F. (2018). Expansion of the *Clostridium perfringens* toxin-based typing scheme. *Anaerobe*, *53*, 5–10. https://doi.org/10.1016/j.anaerobe.2018.04.011

Rood, J. I., & Cole, S. T. (1991). Molecular genetics and pathogenesis of *Clostridium perfringens*. *Microbiological Reviews*, *55*(4), 621–648. https://doi.org/10.1128/mr.55.4.621-648.1991

Sainsbury, A. W., & Vaughan-Higgins, R. J. (2012). Analyzing disease risks associated with translocations. *Conservation Biology*, *26*(3), 442–452. https://doi.org/10.1111/j.1523-1739.2012.01839.x

Schoster, A., Arroyo, L. G., Staempfli, H. R., Shewen, P. E., & Weese, J. S. (2012). Presence and molecular characterization of *Clostridium difficile* and *Clostridium perfringens* in intestinal compartments of healthy horses. *BMC Veterinary Research*, *8*(1), 1–6. https://doi.org/10.1186/1746-6148-8-94

Silberman, M. S., & Fulton, R. B. (1979). Medical problems of captive and wild rhinoceros: A review of the literature and personal experiences. *The Journal of Zoo Animal Medicine*, *10*(1), 6–16.

Silva, R. O. S., & Lobato, F. C. F. (2015). *Clostridium perfringens*: A review of enteric diseases in dogs, cats and wild animals. *Anaerobe*, *33*, 14–17. https://doi.org/10.1016/j.anaerobe.2015.01.006

Songer, J. G. (1996). Clostridial enteric diseases of domestic animals. *Clinical Microbiology Reviews*, *9*(2), 216–234.

Songer, J. G. (1997). Clostridial diseases of animals. In *The clostridia* (pp. 153–182). Molecular Biology and Pathogenesis. https://doi.org/10.1016/B978-012595020-6/50012-7

Springer, S., & Selbitz, H. J. (1999). The control of necrotic enteritis in sucking piglets by means of a *Clostridium perfringens* toxoid

vaccine. *FEMS Immunology & Medical Microbiology*, *24*(3), 333–336. https://doi.org/10.1111/j.1574-695X.1999.tb01302.x

Stämpfli, R. H., & Oliver-Espinosa, J. O. (2021). *Infectious necrotic hepatitis in animals*. MSD Veterinary Manual https://www.msdvetmanual.com/generalized-conditions/clostridial-diseases

Teixeira, C. P., De Azevedo, C. S., Mendl, M., Cipreste, C. F., & Young, R. J. (2007). Revisiting translocation and reintroduction programmes; the importance of considering stress. *Animal Behaviour*, *73*, 1–13. https://doi.org/10.1016/j.anbehav.2006.06.002

Traub-Dargatz, J. L., & Jones, R. L. (1993). Clostridia-associated enterocolitis in adult horses and foals. *Veterinary Clinics of North America: Equine Practice*, *9*(2), 411–421. https://doi.org/10.1016/S0749-0739(17)30407-8

Tubbesing, U. (2017). *Rhinovax*®. Wildlife Vets Namibia https://wildlifevetsnamibia.com/documentation/

Underwood, W. J., Blauwiekel, R., Delano, M. L., Gillesby, R., Mischler, S. A., & Schoell, A. (2015). Biology and diseases of ruminants (sheep, goats, and cattle). In *Laboratory animal medicine* (pp. 623–694). Academic Press. https://doi.org/10.1016/B978-0-12-409527-4.00015-8

Uzal, F. A., Navarro, M. A., Asin, J., & Henderson, E. E. (2022). Clostridial diseases of horses: A review. *Vaccine*, *10*(2), 318. https://doi.org/10.3390/vaccines10020318

Uzal, F. A., & Songer, J. G. (2008). Diagnosis of *Clostridium perfringens* intestinal infections in sheep and goats. *Journal of Veterinary Diagnostic Investigation*, *20*(3), 253–265. https://doi.org/10.1177/104063870802000301

Uzal, F. A., Songer, J. G., Prescott, J. F., & Popoff, M. R. (Eds.). (2016). *Clostridial diseases of animals*. John Wiley & Sons.

Uzal, F. A., Vidal, J. E., McClane, B. A., & Gurjar, A. A. (2010). *Clostridium perfringens* toxins involved in mammalian veterinary diseases. *The Open Toxinology Journal*, *2*, 24–42.

Vahala, J., Vondra, Z., Kober, M., & PETRŽÍLEK, P. (1995). Handfeeding a black rhinoceros *Diceros bicornis* calf at Dvur Kralove zoo. *International Zoo Yearbook*, *34*(1), 211–217. https://doi.org/10.1111/j.1748-1090.1995.tb00681.x

Vidor, C., Awad, M., & Lyras, D. (2015). Antibiotic resistance, virulence factors and genetics of *Clostridium sordellii*. *Research in Microbiology*, *166*(4), 368–374. https://doi.org/10.1016/j.resmic.2014.09.003

Woodford, M. H., & Rossiter, P. B. (1994). Disease risks associated with wildlife translocation projects. *Creative Conservation*, 178–200. https://doi.org/10.1007/978-94-011-0721-1_9

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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