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CLINICAL FINDINGS, PATHOLOGY, BIOSECURITY, AND SEROSURVEILLANCE OF COXIELLOSIS IN WHITE RHINOCEROSES (*CERATOTHERIUM SIMUM*) AT A CONSERVATION CENTER: TWO CASES

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Abstract: A primiparous white rhinoceros (*Ceratotherium simum*) gave birth to a calf overnight after approximately 16 mo of gestation. The calf was found dead in the morning. Necrosuppurative placentitis with bacterial inclusions suggestive of coxiellosis was diagnosed histologically, and *Coxiella burnetii* was identified in fetal tissues and placenta by polymerase chain reaction and immunohistochemistry. Another primiparous female from the same herd aborted later that year after approximately 15 mo of gestation, and coxiellosis was similarly diagnosed in fetal tissues and on vaginal shedding. Estimates of exposure time, duration of vaginal shedding, and phase I and phase II antibody dynamics were determined retrospectively and prospectively for the two confirmed cases. Biosecurity measures were put in place to prevent guests, staff, and conspecific exposure to the organism. No other confirmed cases have occurred in the collection 3 yr after the initial cases. Coxiellosis outbreaks could represent an emerging threat to conservation efforts and ex situ white rhinoceros breeding programs.

CLINICAL BRIEF

Clinical cases

A 7-yr-old primiparous white rhinoceros (*Ceratotherium simum*; WR1) gave birth overnight to a male calf after 497 days of gestation, which is within the reported gestational length of 16–19 mo.[7] This dam was part of a herd of 10 white rhinoceroses housed permanently outdoors. The calf was found dead at the first morning check. On gross exam, the calf weighed 39.5 kg, suggesting prematurity, and was in good condition. The lungs were partially inflated, suggesting that the calf was alive at birth. Approximately 1.5 L of hemorrhagic effusion was present in the abdominal cavity, with approximately 250 ml in the thoracic cavity. The hemorrhagic fluid had a packed cell volume of 35% and total solids of 6.9 g/L, suggestive of whole blood. The remainder of the gross exam was unremarkable. The placenta was retrieved from the field and looked grossly abnormal. The maternal aspect of the chorioallantois was covered with purulent discharge (Fig. 1). Aerobic and anaerobic bacterial culture from the placenta showed heavy growth of *Bacillus* sp., beta-*Streptococcus* group C and *Burkholderia cepacia*. Fungal culture yielded no growth after 14 days. Aerobic and anaerobic bacterial culture of fetal abdominal fluid showed heavy growth of *Bacillus* sp.

Clinically, the dam was acting normally and had no abnormalities on visual exam. Complete blood count and serum biochemistry results were within reference intervals.[10] For closer monitoring, the dam was kept separated from the rest of the herd and had access to two corrals. The animal was started on an empirical course of trimethoprim-sulfamethoxazole (Amneal Pharmaceuticals LLC, Bridgeport, NJ 08807, USA; 26,880 mg PO q12 hr for 14 days). Vaginal discharge stopped within a few days, and the animal was let back out with the herd as she showed signs of maladaptation to isolation, such as climbing the corral bars and pacing.

Histopathology for WR1 was released 4 mo after parturition and revealed acute, severe necrosuppurative placentitis with intracellular bacterial inclusions in presumed necrotic trophoblasts and macrophages, which was considered the cause for premature birth of the calf (Fig. 2). Multiple hemorrhages were seen throughout the fetus, including in the lungs, presumably from dam-induced or labor-related trauma. Acute congestive heart failure, caused by necrosis of one of the atrioventricular valve leaflets, likely impaired normal systolic function. The cause of valve necrosis is unknown. Possible causes include low-grade sepsis from the placentitis, trauma before death, or

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Figure 1. Photograph of the placenta from WR1 showing the maternal aspect of the chorioallantois covered with purulent discharge.

other causes. Regardless of the initiating cause, valve necrosis of this degree would lead to compromise of valvular function and likely result in heart failure. Gram and Gimenez stains of placental tissue showed strongly Gimenez-positive, gram-negative intracellular bacteria, consistent with either *Coxiella* sp. or *Chlamydia* sp. (Fig. 2D). Polymerase chain reaction (PCR) performed on frozen placenta confirmed the presence of *Coxiella burnetii* DNA and the absence of *Chlamydia* sp. DNA. This result was confirmed by immunohistochemistry (IHC) with anti-*Coxiella* monoclonal antibody immunoglobulin G (mAb IgG)3 8A4 PS-464 antibodies on formalin-fixed paraffin-embedded placental tissue (Fig. 2B).

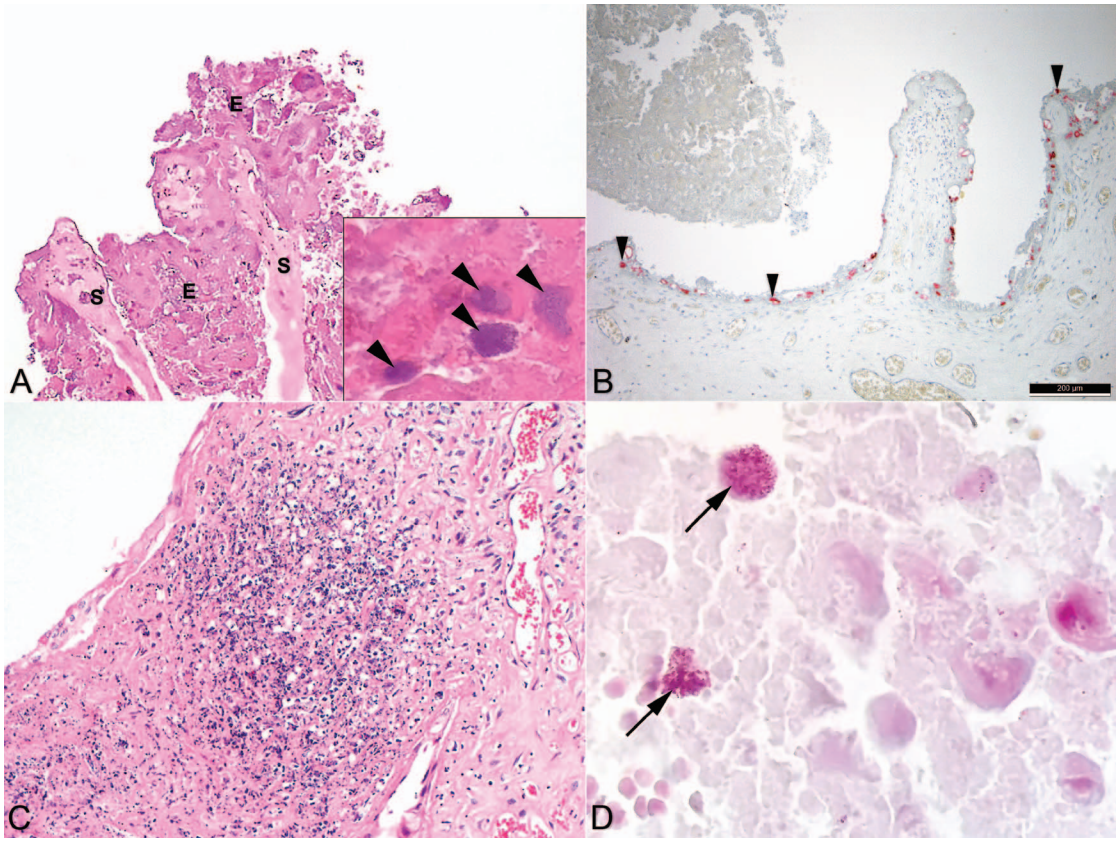

Figure 2. Photomicrographs of the placenta from a white rhinoceros (WR1) infected with *Coxiella burnetii*. **A.** The surface epithelium (E) and subjacent fibrovascular stalks (S) of chorioallantoic villi are necrotic. Inset: Trophoblasts are expanded by intracytoplasmic, basophilic, bacterial microcolonies (arrowheads). H&E; ×100 magnification, inset ×400 magnification. **B.** Trophoblasts have strong, cytoplasmic immunoreactivity evidenced by red chromogen staining in trophoblasts (arrowheads) for *Coxiella* antigen. Anti-*Coxiella* sp. immunohistochemistry with red chromogen; ×40 magnification. **C.** Focus of lytic necrosis in the chorioallantois characterized by necrotic cellular debris, fragmented neutrophils, and mineral. H&E; ×100 magnification. **D.** Necrotic trophoblasts are distended with intracytoplasmic microcolonies of bacteria (arrows) that stain positively for Gimenez. Gimenez stain; ×400 magnification.

Two weeks after the PCR results were available from WR1 and 5 mo after the first case, a 5-yr-old primiparous white rhinoceros (WR2) from the same herd gave birth overnight. The carcass of a female calf was found in the morning and was heavily predated by wildlife. The placenta was not found. The estimated gestation time of this calf was either 462 or 493 days on the basis of observed copulation. On postmortem examination, the lungs of the calf were atelectatic, suggesting stillbirth or abortion. Gross postmortem examination was unremarkable. The calf weighed 30.5 kg. Histologic findings were underwhelming and included renal and cerebellar congestion and mild hepatocellular necrosis with hemorrhage. Because of the recent diagnosis of coxiellosis in WR1, fresh spleen, liver, and lymph node tissues from the calf were submitted individually for *C. burnetii* and *Chlamydia* sp. quantitative PCR, as were a vaginal swab and milk sample from the clinically healthy dam. The fetal samples were pooled and positive for *C. burnetii* DNA (cycle threshold [Ct] value 36.1). The vaginal swab was strongly positive for *C. burnetii* DNA (Ct value 21.5) and negative for *Chlamydia* sp. Monitoring of vaginal shedding was instituted and became negative by day 36 after parturition. *Coxiella* DNA was not detected in the milk sample.

Although coxiellosis is not a notifiable animal disease in the United States, the outbreak was reported to the state veterinarian. An institution-wide notice was sent to all employees to alert them of the presence of the pathogen on grounds. A herd management plan was put in place to reduce risk of exposure and transmission. Figure 3 depicts the different white rhinoceros holding spaces. After WR2's abortion, both WR1 and WR2 were isolated from the rest of the herd in two corrals. The corrals were bordered by a road and the adjacent paddock. Access to the road approaching the corrals was blocked and only used by authorized personnel. The paddock adjacent to their corral was used as a buffer zone, and the rest of the herd was moved to the next paddock over to prevent direct contact with WR1 and WR2. Only one vehicle with a dedicated keeper crew serviced the isolated road, and this was done at the end of the day, after all other animals had been taken care of. While servicing the area, keepers wore personal protective equipment including coveralls, plastic boot covers, gloves, and N95 masks. Keepers were given zoonotic training on Q fever and encouraged to speak to their physicians about potential exposure. WR1 and WR2 were released from isolation once WR2 vaginal shedding had stopped at day 36 after parturition. Minimal animal movement between enclosures has occurred since the initial case to reduce exposure in the other breeding white rhinoceros herds. Guest tours in the area resumed once isolation was completed.

To estimate exposure time, banked serum samples were retrospectively analyzed by immunofluorescence (IFA) for phase I (chronic) and phase II (acute) antibodies against *C. burnetii*. Serum was also submitted prospectively to monitor antibody dynamics postinfection. The results are summarized in Figure 4.

DISCUSSION

Coxiellosis, or Q fever, is a zoonotic disease reported worldwide, caused by the highly infectious gram-negative obligate intracellular coccobacilli *C. burnetii*. Known reservoirs include domestic ruminants, wildlife, and ticks, with a transmission cycle occurring between the latter two.[2,3] Excretion of the organism from infected animals occurs predominantly in birth products, mainly the placenta, but can also be found in feces, urine, semen, vaginal discharge, milk, and sputum.[1] Aerosol inhalation is the most common form of transmission and will occur by contact with birth products or inhalation of contaminated fomites. Along with domestic ruminants, ticks are a known reservoir of *C. burnetii* and serve as vectors in the wildlife transmission cycle.[2,3] Without extensive epidemiosurveillance programs in place for wild animals, the true role of wildlife in dispersion and maintenance of *C. burnetii* remains vastly unexplored, and the increasing number of detection reports may only reflect better recognition of the disease as opposed to emergence of coxiellosis worldwide.[11]

Coxiellosis in domestic ruminants is often subclinical, but when disease is evident, it usually manifests as abortion, stillbirth, endometritis, mastitis, infertility, or a combination of conditions.[1] Chronically infected livestock can present with abortion, low birth weight, and infertility.[6] Little information is available on clinical manifestations of coxiellosis in nondomestic animals, but several molecular studies investigating the presence of *Coxiella* DNA in wildlife and associated ticks are available for review.[2–5,11]

This is the first report of *Coxiella*-related placentitis and stillbirth at this institution and the first confirmed report of the disease in rhinoceroses. Clinically, both dams lacked signs of illness before parturition or abortion, and their

Figure 3. Aerial photograph of the different white rhinoceros holdings at the institution. Both parturition events occurred in H40-45. WR1 and WR2 were isolated from the rest of the herd and housed in two corrals in H45 (white *). The rest of the H40-45 herd were kept in H40 (tree line). The road was blocked from vehicular traffic (red squares), and only one truck was allowed to service the area. Roads separated H40-45 from H46 and H41, so no direct contact between rhino herds was possible.

bloodwork remained unremarkable. However, both dams had extremely elevated phase I and II *C. burnetii* titers at the time of calf delivery. Whereas WR2's calf was stillborn, WR1's calf was alive at birth and, based on the postmortem exam report, likely sustained some trauma during the periparturient period, either because of difficult parturition or postpartum trauma inflicted by the dam. It is not uncommon for inexperienced white rhinoceros dams to rigorously stimulate their young shortly after birth to entice them to get up and nurse. Although this was not observed, the authors suspect the dam might have tried to get her weak calf to rise and could have caused trauma to her offspring, contributing to its death.

Diagnosis of coxiellosis is based on characteristic placental lesions, which include necrosuppurative placentitis with intracytoplasmic bacterial microcolonies; histologic lesions in the fetus are usually minimal, if any.[9] Access to WR1's placenta was of key importance in the diagnosis of this outbreak. Confirmation of the diagnosis can be achieved with PCR, IHC, and IFA.[9] WR1's diagnosis was based on serology, gross necropsy, and histopathologic lesions and was confirmed by PCR and IHC, whereas WR2's diagnosis was based solely on PCR findings, along with clinical presentation and extremely elevated phase I and II *C. burnetii* titers.

The timeline of exposure was not known at the time of diagnosis; therefore, a retrospective and prospective review of *C. burnetii* antibodies was done for WR1 and WR2. During the course of infection, the outer membrane of *C. burnetii*

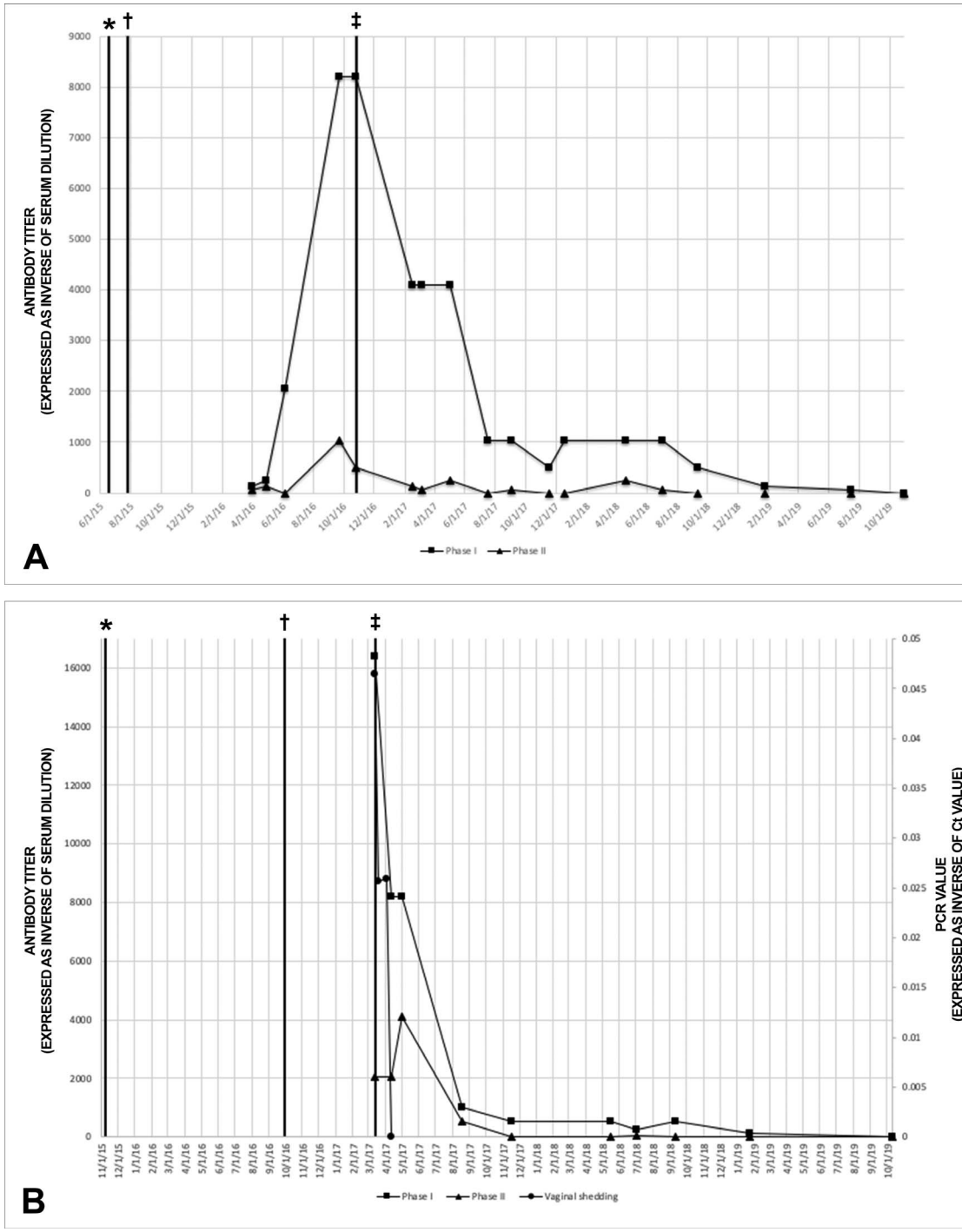

Figure 4. Timeline of WR1 (**A**) and WR2 (**B**) immune response to phase I and phase II *Coxiella burnetii* antigens as measured by IFA. Vaginal shedding as determined by PCR, is also presented in panel B. Vertical lines depict other important time marks: conception time (*), latest known time of seronegativity (†), and parturition (‡).

undergoes changes in its lipopolysaccharide structure, a phenomenon called phase variation.[8] Differences in phase I and phase II antigen presentation can help determine if the infection is acute or chronic. In acute Q fever, the phase II antibody is usually higher than the phase I titer, even in early specimens.[8] Although a rise in phase I as well as phase II titers may occur in later specimens, the phase II titer remains higher. In chronic Q fever, the reverse situation is generally seen. Serum specimens drawn late in the illness from chronic Q fever patients demonstrate significantly higher phase I titers.[8] The latter seems to have been the case for WR1 and WR2 at the time of diagnosis, an unexpected finding assuming that the serology of coxiellosis in rhinoceros is similar to that in livestock and humans. The duration of the acute phase of coxiellosis in white rhinoceros remains unknown and would likely be challenging to detect without controlled experimentation.

In both WR1 and WR2, exposure to *C. burnetii* occurred after conception. Both dams were bred by the same individual, who has consistently been seronegative before and after the outbreak (data not shown). The bull was removed from the breeding herd 2 mo after breeding WR2 and 8 mo after WR1. No *Coxiella* DNA was detected in the sire's semen at approximately 4 mo after WR1's parturition. WR1 was exposed between the first and 10th month of gestation, whereas WR2 was exposed sometime between the 10th and 16th month of gestation (time of parturition), suggesting that exposure at any timepoint during gestation could result in clinical disease (i.e., placentitis or stillbirth). Whether or not clinical disease would occur if exposure happened before conception remains unknown.

The source of *C. burnetii* has not been identified in this case but was most likely local wildlife, as the white rhinoceros herds of this facility are permanently housed outdoors, and wildlife such as white-tailed deer (*Odocoileus virginianus*), wild boar (*Sus scrofa*), raccoons (*Procyon lotor*), Virginia opossums (*Didelphis virginiana*), and various species of birds are commonly seen on the premises. The possibility of an asymptomatic persistently infected rhinoceros cannot be excluded, either, which has triggered the institution to conduct a retrospective and prospective serosurveillance program in the herd. It is unknown if WR1 and WR2 would remain persistently infected and if shedding would reoccur with subsequent pregnancies. Because of the institution's mission of conservation through captive breeding of endangered species, both WR1 and WR2 are highly valuable individuals and will be bred again in the near future. Close monitoring of *C. burnetii* antibodies will be conducted as per the institution's birth management plan.

Biosecurity measures were implemented to reduce risk of exposure to this zoonotic agent for guests and staff and to avoid transmission to other conspecifics. The zoonotic potential of *C. burnetii* is well known, and outbreaks in humans are usually linked to contact with domestic ruminants or their products.[8] Since 2000, Q fever is a reportable disease in humans in the United States. However, coxiellosis is not a reportable disease to the US Department of Agriculture, Animal, and Plant Health Inspection Service. The authors elected to inform the state veterinarian proactively of the infection, because guests and staff could have been exposed to the agent before its diagnosis. Guidelines on prevention and control of *C. burnetii* among humans and animals by the National Association of State Public Health Veterinarians and National Association of State Animal Health Officials were reviewed, adapted, and instituted at the facility. Because of the facility's enclosure design, it was impossible to relocate WR1 and WR2 away from all other rhinoceroses, but it was feasible to avoid direct contact with other white rhinoceros by isolation in corrals. It should be noted that WR1 was put back in contact with the rest of the herd shortly after her parturition and that shedding was not monitored in her case because the diagnosis was not made at the time. On the same note, WR1 and WR2 both gave birth while sharing the same pasture as the rest of the herd. Therefore, exposure to birth products and shedding dams is likely to have occurred, regardless of the biosecurity measures established once the diagnosis was made on WR1. That being said, the institution immediately put in place a birth management protocol for the white rhinoceros herd to minimize subsequent exposure. Details of the serosurveillance program and birth management plan will be described elsewhere.

The authors describe the first confirmed outbreak of coxiellosis in a captive herd of white rhinoceros in North America. Exposure from an unknown source during gestation led to clinical disease. Coxiellosis was confirmed by PCR in both cases. When placenta was available, necrosuppurative placentitis was seen. Serology was extremely valuable in understanding antibody titer fluctuation before and during the outbreak and can be used as a monitoring tool during gestation of other white rhinoceroses with a high

risk of exposure. Vaginal shedding occurred for at least 3 wk in one case. Disease information should be made available to staff working with positive herds, and biosecurity measures should be implemented. Further investigation is needed to fully understand the prevalence, disease, zoonotic risk, and conservation effect of coxiellosis in captive and free-ranging white rhinoceroses.

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