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Case Study

Diagnosis, Surveillance and Management of *Streptococcus equi* subspecies *zooepidemicus* Infections in Chinchillas (*Chinchilla lanigera)*

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During a 6-mo period, two 5-6 mo old female chinchillas (*Chinchilla lanigera*) were examined at the University of Colorado Anschutz Medical Campus after the discovery of firm, nonmobile masses in the left ventral cervical and left axillary region. Other than these findings and mild weight loss, both chinchillas' physical exams were normal. Bloodwork revealed an inflammatory leukogram characterized by leukocytosis, toxic neutrophils, lymphopenia, and monocytosis with mild, nonregenerative anemia. At necropsy, both masses were identified as abscesses. *Streptococcus equi*, subspecies *zooepidemicus* (*S. zooepidemicus*) was isolated in pure culture. Histology of the lungs, liver, spleen, and kidneys showed a marked increase in the numbers of both polymorphonuclear leukocytes and lymphocytes. Both animals were deemed unsuitable for research and were euthanized under isoflurane anesthesia by an intracardiac injection of pentobarbital sodium solution. *S. zooepidemicus* is an opportunistic, commensal organism found in the upper respiratory tract of horses. This organism has been documented to cause disease in other species and is zoonotic. Infections in humans have been reported, resulting in glomerulonephritis, endocarditis, septic arthritis, osteomyelitis, meningitis, and death. To aid in diagnosis and prospective surveillance of this bacteria, oral and nasal swabs were collected from the remaining cohort of chinchillas, and a qPCR screening assay was implemented. Within 12 mo, 4 of 41 additional females tested positive by culture or qPCR, resulting in a disease prevalence of 14% (6 of 43). However, only 2 of the additional 4 *S. zooepidemicus* positive animals developed clinical signs. The potential for the spread of infection, zoonosis, and adverse effects on research demonstrate that surveillance for *S. zooepidemicus* should be considered in a biomedical research environment.

Abbreviations: PMNs, Polymorphonuclear leukocytes; *S. zooepidemicus, Streptococcus equi* subspecies *zooepidemicus;*

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S. *zooepidemicus* is a gram-positive, opportunistic, β-hemolytic, Lancefield group C bacterium.[9,23] This pathogen has been found to cause disease in a variety of animals, including horses, pigs, chickens, monkeys, ruminants, cats, dogs, guinea pigs, and humans.[2,8,12,14,20,21,34] In horses it is one of the most common commensal organisms found in the upper respiratory and urogenital tracts, as well as on the skin.[7,9,23] Although more often caused by *Streptococcus equi* subsp. *equi*, *S. zooepidemicus* has been isolated alone in cases of strangles (a well-known disease of horses) in immune-compromised horses.[23] Whether an infection in horses is caused by *S. equi* subsp. *equi* or *S. zooepidemicus*, the disease typically presents with clinical signs of pyrexia, swollen or abscessed lymph nodes, serous to purulent nasal discharge, anorexia, coughing, pneumonia, and depression.[7,11,23,24] This pathogen is usually transmitted via oral and nasal routes, but may be transmitted by skin contact[31] and contaminated food.[3,6,19]

Regarding the zoonotic potential of *S. zooepidemicus* infection, several cases of zoonosis from horses, guinea pigs, and dogs have been documented.[1,9,13,14,16,27] In 2013, 2 human cases of septic shock and multiple organ failure were found to be due to *S. zooepidemicus* transmission from pet guinea pigs.[31] More recently, the Department of Pediatric Infectious Diseases at the University of Texas reported a case of meningitis in a 6-mo infant that tested positive for *S. zooepidemicus*.[35] In humans, severe infections causing glomerulonephritis, endocarditis, pneumonia, pericarditis, septic arthritis, osteomyelitis, meningitis, and death have been reported.[1,13,18,31,35]

Case Study

Clinical case 1. A 5-mo old, 415 g intact female chinchilla (*Chinchilla lanigera)* was received at our institution on June 29, 2017 for use in an acoustic and physiologic measurement research project. Physical examination at the time of arrival was normal. Forty days later, the experimentally naive chinchilla was evaluated for mild inappetence, weight loss, and a large left submandibular swelling (Figure 1 A). On examination, the

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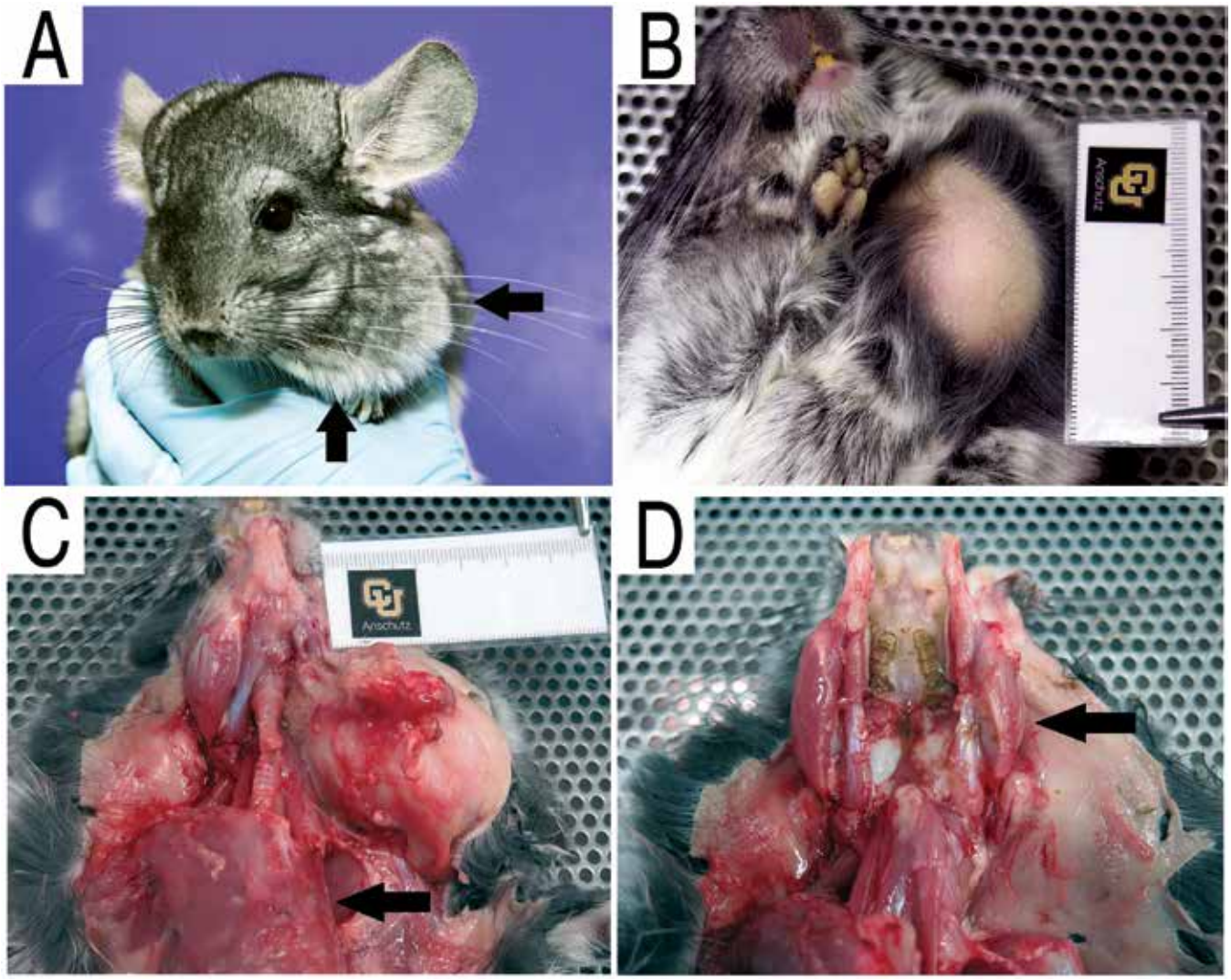

Figure 1. Antemortem and postmortem picture of clinical case 1. (A) The gray gradients of dense and lanate hair coat of the chinchilla effectively obscured the development of the abscess (between arrows) from identification by routine visual evaluation. (B) In dorsal recumbency, alopecia of the dependent aspect of the mass, without noticeable skin inflammation, irritation, or abrasion was a common finding for abscesses of similar size. (C) Necropsy image, in dorsal recumbency with skin removed and encapsulated abscess exposed. The arrow denotes the lack of a deep pectoral muscle in the left axilla. (D) Removal of the mandible and in block removal of the well encapsulated abscess noted atrophy to the left masseter and digastricus muscles (arrow). The dental arcade was within normal limits.

chinchilla was bright, alert, and responsive, had a body condition score of 2.5/5, and temperature, pulse, and respiration were all within normal limits. On palpation, the left submandibular mass was firm, nonmobile, and nonpainful. The animal was euthanized due to this finding, which made it a poor research candidate. Before euthanasia, the animal was anesthetized in an induction chamber with 3% isoflurane at a flow rate of 2 L/min of O_2. The animal was transferred to a nose cone to maintain anesthesia, and 1 mL of blood was drawn from the left femoral vein for a CBC. Next, euthanasia was performed by using a 2 mL intracardiac injection of pentobarbital sodium solution (Fatal-Plus), and a diagnostic necropsy was performed.

Upon visual inspection, the submandibular mass measured 4 x 3 x 3 cm. Margins extended to the lower left mandible, dorsal left scapular region, and the left ventral area of the thorax near the sternum (Figure 1 B). Exposure of the thoracic muscle layers revealed a lack of deep pectoral muscles on the left side below the mass (Figure 1 C). The mass was dissected out, showing atrophy of the left masseter and digastricus muscles (Figure 1 D). The right side contained a much smaller mass that was initially thought to be the right salivary gland. This mass was also dissected out for comparison. No other gross lesions or abnormalities were seen. Upon sectioning, both masses contained white, caseous exudate and were identified as abscesses (Figure 2). The left abscess contained approximately 9.0 mL of white caseous exudate. This exudate was collected with sterile swabs and sent to IDEXX BioAnalytics for aerobic culture and identification. Tissue samples were collected from both abscesses, lungs, liver, kidney, and spleen for histopathology.

The CBC results indicated that the chinchilla had mild, nonregenerative anemia, likely due to an inflammatory response (anemia of inflammatory disease) as characterized by leukocytosis, lymphopenia, monocytosis, and toxic neutrophils. *S. zooepidemicus* was isolated in pure culture from the mass, as identified by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS). Histologically, a gram stain was performed, revealing gram-positive cocci in chains. The left abscess was fully contained in a fibrous connective tissue capsule that incorporated the thyroid and a portion of the parotid salivary gland (Figure 3). Fatty tissue surrounding the

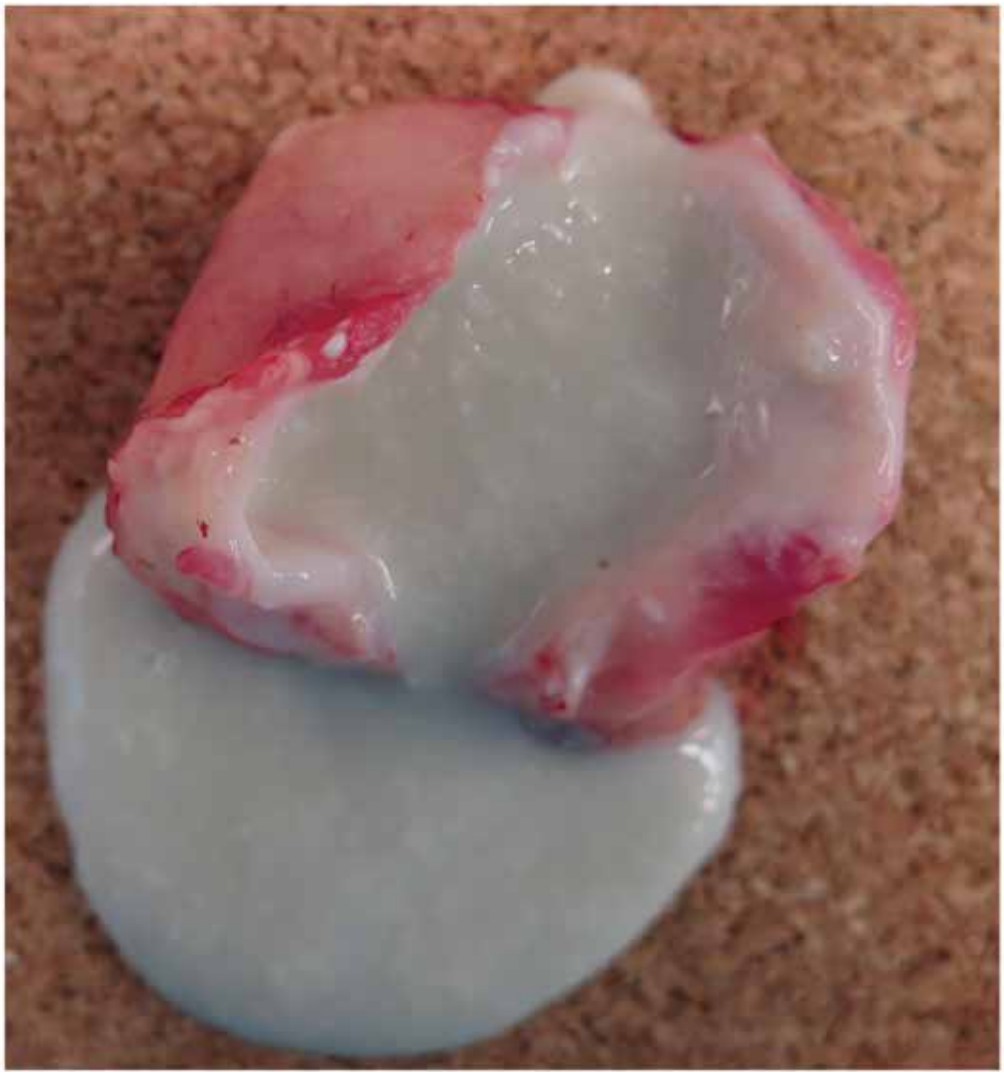

Figure 2. Cut section of the left submandibular abscess from clinical case 1 containing 9.0 mL of pearl white caseous exudate. The white color of the purulent material within the abscess was consistent with all other clinical cases.

thymus contained lymphocytes and scattered PMNs. The tissue collected from the lungs, liver, spleen, and kidneys showed the presence of more lymphocytes, PMN proliferation, and macrophages, as well as tubular dilation with leukocytes within the renal tubules.

Clinical case 2. On December 13, 2017, a 6-mo old, 436 g intact female chinchilla was received for the same research project as the case above. Physical examination at the time of arrival was normal. Seventy-six days later, the chinchilla presented with 2 large left axillary masses. Unlike the first case, this chinchilla had undergone several weeks of acoustic behavioral training. On examination, the chinchilla was bright, alert, and responsive, had a body condition score of 2.5/5, and temperature, pulse, and respiration were all within normal limits. On palpation, one mass was craniodorsal to her left axillary area, and the other mass was cranial ventral to her left axillary area. They were both firm, nonpainful, nonmobile, and attached to the muscle of her left forelimb, dorsal thoracic, and dorsal scapular region. As before, euthanasia was elected by the research laboratory due to the animal being a poor research candidate.

Before euthanasia, the animal was anesthetized in an induction chamber with 3% isoflurane at a flow rate of 2 L/min of O_2. To maintain anesthesia, the animal was transferred to a nose cone, and 1 mL of blood was drawn from her left femoral vein for a CBC. Euthanasia was then performed by using a 2 mL intracardiac injection of pentobarbital sodium solution (Fatal Plus). A necropsy was then performed.

On visual inspection, the dorsal cranial abscess measured 3 × 3 x 3 cm, and the ventral cranial abscess measured 2 × 3 x 3 cm. They were joined by connective tissue under the left axillary region, with margins extending to the ventral cranial thoracic to the dorsal cranial region, caudal to the scapula. No other gross lesions were present. The 2 masses were dissected out from the left thoracic pectoral muscles, biceps brachii of the left forelimb, and the latissimus dorsi caudal to the scapula. No muscle atrophy was seen on this chinchilla, as compared with the first case. No further lesions or abnormalities were seen. Upon incision of the masses, white caseous exudate was present. The exudate was collected with sterile swabs and sent to IDEXX BioAnalytics for aerobic culture, identification, and antibiotic sensitivity testing. Tissue samples were collected from the mass, lungs, liver, kidney, brain, and spleen and were submitted for histopathology.

The CBC revealed a mild nonregenerative anemia and an inflammatory response with significant hypochromasia and a predominance of target cells and anisocytosis based on published reference values.[29] Leukocytosis, with 3+ toxic neutrophils and

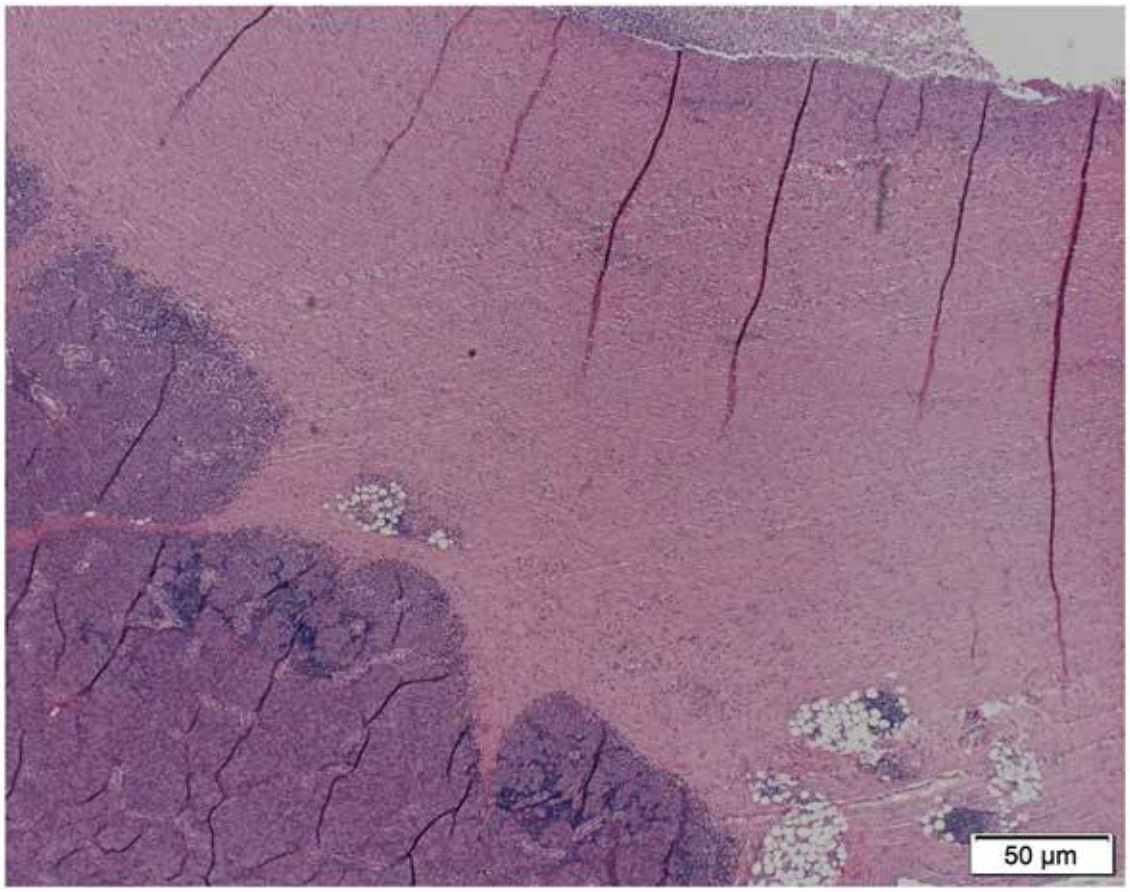

Figure 3. Histological section of the thick, fibrous, eosinophilic capsule wall of the submandibular abscess from clinical case 1, with a marginated portion of the basophilic, parotid salivary gland.

vacuolated monocytes, was also present. As identified by Matrix-Assisted Laser Desorption Ionization Time-Of-Flight Mass Spectrometry, *S. zooepidemicus* grew in pure culture from the samples of purulent material collected. The fibrous capsule of the abscess contained multifocal pockets of activated macrophages and neutrophils, with occasional foci of lymphocytes noted. Coagulative necrosis was seen within muscle cells that were attached to the fibrous capsule of the abscess with fibroblasts, macrophages, and poorly organized neutrophils. Marked amounts of PMNs and macrophages were seen within the choroid plexus, heart, thyroid, and lungs. Within the liver, there was oval cell hyperplasia, with a marked increase in the number of infiltrating lymphocytes and PMNs.

Case-related Husbandry Chinchillas were obtained from the Ryerson Chinchilla Ranch (Plymouth, OH), which supplies chinchillas for both research and the pet-trade. Chinchillas at our institution are pair-housed in Allentown rabbit caging (4.5ft^2 floor space, 28″x 23″x 17″) on a 12:12 h light: dark cycle. Chinchillas are fed Mazuri Chinchilla Diet (Mazuri Exotic Animal Nutrition, St Louis, MO) and supplemented with timothy hay and daily nutritional enrichment of dried fruits and nuts. Dust baths are provided 3 times per week. The chinchillas are housed in the same room as nonspecific pathogen-free guinea pigs (*Cavia porcellus*), but all supplies are physically and procedurally separated. Standard personal protective equipment for entering this room includes a disposable gown, hair bonnet, and the use of gloves during handling. All animals were euthanized using acceptable methods from the AVMA Guidelines for the Euthanasia of Animals.[22] Chinchilla manipulations and procedures were approved by the University of Colorado Denver, Anschutz Medical Campus IACUC and were performed in an AAALAC-accredited facility.

Bacteriology and Molecular Diagnostic. Methods: Culture swabs collected from both cases at the time of necropsy were aseptically removed from the transport medium and streaked for isolation onto BBL Trypticase Soy Agar with 5% sheep blood (TSA II; Becton Dickinson). Culture plates were incubated aerobically at 35 °C with 7% CO_2. Representative colonies were selected and harvested for proteomic analysis using the direct transfer method as previously described.[10,28] Bacteria were overlaid with 1 µL of a saturated solution of α-cyano-4-hydroxycinnamic acid in 50% acetonitrile, 2.5% trifluoroacetic acid (Matrix HCCA, Bruker Daltronics, Billerica, MA), allowed to dry, and analyzed by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) using a mass spectrometer (Microflex, Bruker Daltronics) and flexControl software (Bruker Daltronics). Bacterial identification was based on automated analysis by MALDI BioTyper software (Bruker Daltronics), which compared the spectra for each isolate with an integrated reference spectral database. Antibiotic susceptibility was determined by Kirby-Bauer disk diffusion assay for *S. zooepidemicus* isolated from the second case. The isolate showed antibiotic sensitivity to Amikacin, Amoxicillin/Clavulanic Acid, Azithromycin, Cefazolin, Ceftiofur, Chloramphenicol, Enrofloxacin, Erythromycin, Gentamicin, Sulfamethoxazole/Trimethoprim, Tetracycline, and Tulathromycin.

To aid in the diagnosis and surveillance of *S. zooepidemicus*, a qPCR assay was implemented based on specific primers and probes for sodA and seeI to differentiate *Streptococcus equi* subsp. *equi* from *Streptococcus equi* subsp. *zooepidemicus*, as

described by Baverud and colleagues 2007.[4] Quantitative PCR was performed in a total reaction volume of 20 µL in 96-well optical reaction plates using 1× TaqMan Buffer A (500 mM KCl, 100 mM Tris-HCl, 0.1 M EDTA, 600 nM passive reference dye ROX; pH 8.3 at room temperature), 300 µM each of dATP, dGTP, and dCTP, 600 µM dUTP, 5.5 mM MgCl2, 500 nM forward primer, 500 nM reverse primer, 200 nM probe, 0.25 U of AmpErase uracil-N-glycosylase, 1.25 U AmpliTaq Gold DNA Polymerase and 10 µL of template DNA . The amplification conditions for the ABI 7900 (Applied Biosystems) consisted of an initial step of 2 min at 50 °C and AmpliTaq Gold activation for 10 min at 95 °C followed by 40 cycles of 15 s 95 °C, 1 min 60 °C. Standard curves were generated by using the fluorescence data from the 10-fold serial dilutions of sodA and seeI DNA amplicons. The standard curve was then used to calculate the absolute copy number of the targets in test samples. Using this assay, all of the remaining 11 chinchillas from the original cohort that accompanied clinical case 1 were tested via PCR by an oral and a nasal swabs collected form each animal.

Results and Management: Of the 11 remaining chinchillas, 2 (18%) more tested positive by PCR for S*. zooepidemicus* by oral swabs only, suggesting exposure, but they never developed clinical evidence of infection. This resulted in a total of 4 of 28 (14%) testing positive for *S. zooepidemicus*, with only 2 (7%) developing abscesses after arriving at the institution. Because *S. zooepidemicus* has been documented to cause chronic lymphadenitis in guinea pigs,[12,33] oral and conjunctival swabs were also collected from the 8 other guinea pigs housed in the same room. All of these guinea pig samples were negative for *S. zooepidemicus*.

To prevent spread of *S. zooepidemicus* within the vivarium, staff were required to change gloves between handling individual chinchillas and to work with guinea pigs prior to working with chinchillas. Upon receiving results from IDEXX BioAnalytics, our institution's occupational health and safety program was notified, and precautions were taken by all animal care staff and lab members that entered the chinchilla housing room. In addition to standard personal protective equipment (disposable gowns, hair bonnet, and gloves), signs were placed to notify staff and lab members of the zoonotic potential and to recommend changing gloves between each chinchilla. All staff were required to wash hands after leaving the room. Only one shipment of chinchillas at a time was allowed at our institution to allow time for proper decontamination of the room before the arrival of new chinchillas.

After ruling out the guinea pig population as the source of infection, the chinchilla vendor was contacted to aid in the identification of the source of the infection. At that time, the vendor confirmed that subcutaneous abscesses, from which *S. zooepidemicus* had been cultured, and had seen previously in their breeding colony and that these lesions had usually been treated by administering antibiotics and lancing the abscess. Identification of the cause and source of abscesses were not given by the vendor during this communication. Given our controlled research environment, standard PPE requirements, and PCR testing of our guinea pigs, we speculate that our chinchillas acquired the pathogen prior to the arrival at our institution. In an uncontrolled environment, potential sources of *S. zooepidemicus* such as contaminated feed and transmission from other domesticated species is possible and should be considered.

As a result of these findings, a surveillance program was initiated using oral swabs as the primary sample for all chinchillas arriving at the institution. Since August 2018, 2 additional chinchillas euthanized and confirmed infected with *S. zooepidemicus* out of a cohort of 15 delivered from the vendor (13%). One presented with a subcutaneous mass on the ventral aspect of the neck and the other with respiratory depression, which was later confirmed as a septic pleural effusion where *S. zooepidemicus* was cultured. Duration from arrival to clinical presentation ranged from 12 to 76 d (35 ± 30 d, $n = 4$). To date, all chinchillas received after August 9, 2018, have been PCR-negative for *S. zooepidemicus* by oral swab, and no chinchillas have developed clinical signs suggestive of infection.

Discussion

In biomedical research, chinchillas are primarily used for studies related to the ear.[33] Compared with other rodents, chinchilla's large bulla, wide tympanic membrane, and physiology are more comparable to humans than are other small animal models.[30,33] Chinchillas have also been used in studies of immunology, infectious diseases, ophthalmology, and obstetrics.[15,17,25,26,33] The veterinary medicine community has produced relatively few publications or documentation about diseases in chinchillas, and most of the available information has been obtained from the pet and fur trade of chinchillas.[30,33]

S. zooepidemicus has only recently been reported to infect chinchillas. A 2019 publication described a single case of a pet chinchilla presenting with similar clinical signs of a mid-cervical mass and testing positive for *S. zooepidemicus*.[5] The results of our antibiotic susceptibility testing and data published in 2019 are in agreement with a prior publication showing that an antibiotic can be used successfully to treat the infection.[5] In contrast to the 2019 case, our chinchillas were euthanized due to possible research implications, personnel safety, and the potential for cross-contamination and spread to other species within our facility.

In summary, *S. zooepidemicus* pathogen can be found in many research and companion animal species. As with other infectious and zoonotic pathogens, we must take appropriate precautions for the wellbeing of the research animals and personnel. Given this possibility together with the potential adverse effects of *S. zooepidemicus* on animal health and research objectives, establishing programs for adequate health surveillance and monitoring is essential.

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