Corynebacterium pseudotuberculosis as a pathogen of the reproductive tract of male small ruminants: case study and review

Jamie L. Stewart, Miranda D. Vieson, Clifford F. Shipley
Department of Veterinary Clinical Medicine, College of Veterinary Medicine, University of Illinois Urbana-Champaign, Urbana, IL

Summary

Three cases are presented herein describing manifestations of *Corynebacterium pseudotuberculosis,* the causative agent of caseous lymphadenitis (CL), in the reproductive tract of two bucks and a ram. The two bucks were presented for poor sperm quality and were subsequently diagnosed with bilateral suppurative orchitis (Case 1) and epididymitis (Case 2) from which *C. pseudotuberculosis* was cultured. Case 3 is a ram that was presented for semen cryopreservation followed by euthanasia. Electroejaculation produced a poor quality ejaculate, whereas epididymal sperm harvest yielded a better quality sample. Abscesses located adjacent to the epididymis and sigmoid flexure of the penis from CL may have prevented normal ejaculation. All cases presented progressive testicular degeneration, which could result indirectly from exotoxins produced by *C. pseudotuberculosis* or directly from abscess formation. These cases highlight the need to test for and eradicate CL, as it may have deleterious effects on reproductive success in small ruminant breeding farms.

Keywords: Caprine, epididymitis, infertility, oligozoospermia, orchitis, ovine

Background

Corynebacterium pseudotuberculosis is a contagious bacterial agent that causes CL in both sheep and goats.[1] This disease has long been known to cause significant financial loss due to increased mortality related to internal lesions and carcass condemnation related to external lesions.[1] It is both time consuming and costly to eradicate CL from a herd, and therefore, many owners chose to live with it. An early study reported an 8% prevalence of CL abscesses confined to the scrotal fascia of rams with no effect on semen quality.[2] However, the effect of this pathogen on reproductive soundness is becoming increasingly more obvious, even when no abscesses are present.[3] This report includes three cases of CL in small ruminants that highlights the need to eradicate *C. pseudotuberculosis* within small ruminant breeding facilities due to its potentially dire herd health consequences and widespread effects throughout the reproductive tract.

Case 1: orchitis in a buck

Case presentation

An 8-year-old Boer buck was presented to the University of Illinois Veterinary Teaching Hospital with a one month history of an enlarged left testicle and oligozoospermia noted by the owners during routine semen collection. The owners also stated that the buck had a chronic cough for an unknown amount of time. On physical examination, generalized muscle atrophy was apparent with a body condition score of 2/9.[4,5] Bilateral mucoid nasal discharge was present, and increased intensity of lung sounds were audible throughout the entire left thorax. The left testicle was noticeably and asymmetrically larger than the right testicle and firm on palpation (Figure 1). Both epididymides were palpably normal. A complete blood cell count revealed active suppurative inflammation (severe neutrophilia: 24.3×10^3 /µL, reference range $1.2\text{-}7.2\times10^3$/µL; hyperfibrinoginemia: 504 mg/dL, reference range 100-400 mg/dL). Serum chemistry revealed hperglobulinemia (6.0 g/dL, reference range 2.7-4.1 g/dL).

Semen was collected by electroejaculation for analysis. A 31.75 mm diameter, three-electrode ram probe (Lane Manufacturing, Inc., Denver, CO) was placed rectally, and a programmed cycle was run on the electroejaculator unit (Pulsator IV, Lane Manufacturing, Inc., Denver, CO). Grossly, the semen appeared to be mildly cloudy and yellow-colored. Microscopic evaluation revealed oligozoospermia and asthenozoospermia with a subjective total motility of 10% and negligible progressive motility.

Morphologic analysis of the spermatozoa revealed 57% normal sperm cells, 7% simple bent tails, 14% detached heads, 6% proximal droplets, 3% mid-piece defects, and 12% strongly folded tails.

Thoracic ultrasonography revealed mild pleural and pericardial effusion (Figure 2) with a normal heart. Cytological analysis of the pleural fluid revealed a modified transudate with no visible bacteria. Ultrasonography of the right testicle detected 5 and 15-mm round hypoechoic nodules filled with numerous hyperechoic foci (Figures 3A and B). Multiple similar hypoechoic nodules with multiple hyperechoic foci were also visible within the left testicle (Figures 3C and D). These nodules likely represent abscesses consistent with multifocal suppurative orchitis, whereas the hyperechoic foci within the parenchyma suggest mineralization and testicular degeneration.[6]

Differentials for suppurative orchitis in small ruminants included chlamydiosis (chlamydophilosis),[7] specific pyogenic bacteria (e.g., *Trueperella pyogenes*), opportunitistic bacteria (e.g., *Actinobacillus seminis*, *Mannheimia haemolytica*, *Bibersteinia trehalosi*, *Pasteurella multocida*), brucellosis, (*Brucella mellitensis* or *Brucella ovis),* and viruses (sheep or goat pox viruses, lentiviruses, *Bluetongue virus*).[8] *Brucella mellitensis* has been reported once as a cause of orchitis in a buck determined by serum antibodies and testicular culture.[9] While *Brucella ovis* commonly causes epididymitis in rams, experimental infection has led to the development of titers and excretion of the bacteria in semen of goats.[10] Due to herd health concerns, the owner elected to have a *Brucella ovis* serum ELISA performed, which was negative.

Fine needle aspiration was performed on both testicles for culture and cytology. Cytology revealed suppurative inflammation with marked necrosis within both testicles. Bacterial culture revealed growths of *Staphylococcus xylosus* from the left testicle and *Corynebacterium pseudotuberculosis* from the right testicle. Bacterial culture of the semen yielded *S. xylosus* and *Bacillus* sp. Both *S. xylosus* and *Bacillus* sp. isolates in this case were considered incidental contaminants, as they have previously been isolated from both scrotal skin and prepuce in rams without clinical disease.[11]

Outcome

This buck was given a grave prognosis for recovery of breeding ability based on ultrasonographic findings that indicated the efferent ducts were likely destroyed by the abscesses. The overall poor condition of the buck also warranted euthanasia due to welfare concerns. The owner wished to pursue cloning options for the buck, so he was discharged to be euthanized on the farm by the referring veterinarian.

Case 2: epididymitis in a buck

Case presentation

A 4-year-old Boer buck was presented to the University of Illinois Veterinary Teaching Hospital with a primary complaint of asthenozoospermia and subfertility. The buck was purchased by the owners four months previously, but the owners noticed that most does he was observed breeding did not become pregnant. The owners did a semen evaluation at home and estimated ~30% motility.

Generalized physical examination was unremarkable with an adequate body condition score of 5/9.[4,5] Examination and palpation of the scrotum revealed bilaterally enlarged epididymides and small, soft testicles. Ultrasonography revealed flocculent material within the epididymides with multiple, small, hyperechoic foci (Figure 4). The testicles appeared to be undergoing bilateral degeneration and mineralization as determined by the presence of multiple hyperechoic foci within the testicular parenchyma (Figure 4).[6] Transrectal ultrasonography was used to evaluate the accessory sex glands and did not reveal any abnormalities.[12] Differentials for epididymitis in small ruminants include those described above for orchitis, with brucellosis, *A. seminis*, and *Histophilus somni* reported most commonly.[13]

Semen was collected using electroejaculation as described in Case 1. The ejaculate was clear and tested weakly positive (ca. 25) on a leukocyte esterase strip, indicating the presence of leukocytes. The sample was smeared on a slide, stained with a modified Wright Giemsa stain (JorVet Dip Quick Stain,

Jorgensen Labs, Loveland CO)), and evaluated with light microscopy. No spermatozoa were seen, but small numbers of neutrophils were noted (Figure 5). The remainder of the ejaculate was submitted for aerobic and anaerobic culture and Brucella spp. multiplex PCR. Brucella PCR was negative, and culture results are summarized in theTable. The most relevant findings were isolation of both *Corynebacterium pseudotuberculosis* and *Fusobacterium necrophorum* from the ejaculate. While the latter has rarely been implicated as a cause of male reproductive disease,[11] it has been associated with late term abortion in does and ewes.[14,15]

Outcome

Due to the severity of the epididymal lesions and testicular degeneration, this buck was given a poor prognosis for recovery of breeding ability. The growth of two significant pathogens from the ejaculate raised the concern for venereal transmission. Since the buck serviced several does, recommendations were made to test the herd for CL and cull any positives. Due to the high-quality genetic line, the owners instead elected to monitor the herd and opt to test if further attempts to breed the does were unsuccessful.

Case 3: peri-sigmoid flexure abscess in a ram

Case presentation

A 2.5-year-old Montadale ram presented for semen collection and subsequent euthanasia due to progressive and severe musculoskeletal defects. Necropsy later defined these defects as degenerative joint disease (multiple joints) and ankylosing spondylosis of the thoracic vertebrate.

Semen collection was performed using electroejaculation as described in the previous cases. The buck produced 3 mL of moderately cloudy ejaculate with a concentration of 163 million spermatozoa/mL. Microscopic evaluation revealed 50% overall motility. Morphological assessment using a phase contrast microscope at 100× determined that there were 33% normal spermatozoa, 27% primary defects (13% proximal droplets, 9% strongly folded, 5% midpiece defects), and 40% secondary defects (19% simple bent, 12% distal droplets, 8% tailless, 1% abnormal acrosome). Six 0.5-mL straws were produced from this collection with a dose of 81.5 million sperm/straw.

After euthanasia, the testicles were removed and submitted for epididymal sperm harvesting. The caudal epididymis and remaining vas deferens were excised from the tract. The cauda epididymis and vas deferens were flushed with Optixcell extender (IMV Technologies, Maple Grove, MN). A total of 3 mL of well-concentrated (1810 mill/mL) semen was obtained with motility of 90%. Since this was an epididymal collection, morphological analysis was not performed. The ejaculate was extended to a final dose of 200 million sperm/straw and yielded 23 0.5-mL straws. One straw was thawed in a 37°C water bath for 30 sec and found to have a post-thaw motility of 70%. During this procedure, an abscess was observed around one cauda epididymis, but did not penetrate into the lumen (Figure 6A). The caseous material was submitted for aerobic culture, and no bacteria were recovered.

On postmortem evaluation, a 4×3×3 cm abscess was identified adjacent to the sigmoid flexure of the penis, which yielded a moderate growth of *Corynebacterium pseudotuberculosis* (Figure 6B). Grossly, the testicles were asymmetrical. The cut surface of the smaller testicle revealed multifocal yellow to light brown, granular, slightly firm areas scattered throughout the testicle and a thickened, firm epididymis (Figure 7A). In the smaller testis, histopathology revealed testicular atrophy and degeneration with fibrosis, ductular loss, and ectasia of the epididymis (Figure 7B). Brucella spp. multiplex PCR of the testis and epididymis were negative, and culture yielded light to very rare growths of gram negative bacteria interpreted as contaminants (*Enterococcs hirae*, *Escherichia coli*, Beta *Escherichia coli*).

Discussion

Caseous lymphadenitis is a disease of sheep and goats characterized by abscess formation and caused by *Corynebacterium pseudotuberculosis*. *Corynebacterium pseudotuberculosis* has also been diagnosed as a cause of unilateral orchitis and epididymitis in a stallion.[16] While the decreased

reproductive fitness in these three cases seem to be related to the CL abscesses, Mahmood et al. recently reported that an exotoxin produced by *C. pseudotuberculosis*, phospholipase D, is also a major contributor to reduced semen quality, independent of abscess formation.[3] Bucks inoculated with *C. pseudotuberulosis* developed abscesses and enlarged lymph nodes at the site of inoculation (subaxillary region), as expected.[3] In bucks treated with only phospholipase D endotoxin, there was a lack of abscess formation.[3] However, despite the lack of gross lesions, there was a significant decrease in systemic testosterone and an increase in dead and abnormal sperm cells similar to that seen in the bucks inoculated with *C. pseudotuberculosis*.[3] Furthermore, these findings in both treatment groups, when compared to untreated controls, coincided with varying degrees of testicular degeneration and necrosis after three months.[3] Based on this study, it is possible that the testicular degeneration observed in all three cases were primarily a sequela to phospholipase D endotoxin release. Additionally, though post-thaw sperm motility in case 3 was acceptable, *in vivo* fertility should be evaluated as phospholipase D may prematurely induce sperm hyperactivation.[17] These results, in addition to the findings in this case series, highlight a major concern for breeding populations with endemic CL, whether or not there are external lesions.

Bacterial culture of the ejaculate in case 2 yielded for *C. pseudotuberculosis*, which raises concerns about potential venereal transmission. As is evident in case 3, clinicians are commonly presented with animals for semen cryopreservation with unknown disease status. Due to the addition of broad-spectrum antibiotics to extenders, bacterial culture is not routinely performed on semen intended for artificial insemination. Though the prevalence of CL varies greatly by region, information on its prevalence and transmission through semen is lacking.[18,19] Though the semen itself was not cultured, necropsy results indicated that no significant pathogens were isolated from the epididymal mass, testis, or epididymis in case 3, nor were pathogens isolated in case 1. Regardless, the pathogen isolation observed in case 2 should present concerns in regards to transmission of this pathogen when used for artificial insemination. Judson and Songer reported the *in vitro* susceptibility of *C. pseudotuberculosis* to a variety of commonly used antibiotics that include penicillins, macrolides, tetracyclines, cephalosprins, lincomycin, and rifampicin.[20] Though the incorporation of broad-spectrum antibiotics into commercial semen extenders should mitigate transmission concerns, further work may be necessary to ensure that it is sufficient to prevent transmission *in vivo*. Additionally, future studies need evaluate the concentration of phospholipase D in semen of CL-positive animals and ascertain if venereal transmission of these exotoxins can be harmful within the female.

Caseous lymphadenitis has been implicated as a cause of abortion and neonate illness in horses by Poonacha and Donahue[21] and in sheep by Dennis and Bamford.[22] In Boer does, both intranasal and oral inoculation with *C. pseudotuberculosis* altered progesterone and estrogen production following estrus synchronization[23] which may contribute to infertility. A subsequent study by this group found that 30 days after intradermal, intranasal, or oral inoculation, there were varying degrees of necrosis, congestion, inflammatory cell infiltration, and edema within the ovaries, uterus, and iliac lymph nodes.[24] Though he long-term effects of inoculation on female fertility have not been evaluated, they do present concern for a potential naïve herd bred by a new, infected male. Alonso et al evaluated timing of inoculations in Manchega sheep and found that three of four ewes inoculated seven days before mating had normal pregnancies.[25] The remaining ewe had a prolonged gestation with a lamb that died at 26 days of age with *C. pseudotuberculosis* isolated from its liver, heart, and kidney.[25] In all ewes inoculated during the embryonic stage (gestational day 23), abortions, stillborns, or ill-thrift/weak lambs were reported.[25] The last group was inoculated during the fetal stage (gestational day 103) and produced apparently normal lambs.[25] These results provide further evidence that introduction of this contagious pathogen either during mating or in the early embryonic stages can be detrimental to herd reproductive success. Further studies are warranted to better understand the pathology of different strains of *C. pseudotuberculosis* within the female reproductive tract and determine if phospholipase D production is responsible for interfering with conception and pregnancy. Understanding these underlying mechanisms is crucial for developing better preventative and intervention strategies for this highly infectious and detrimental disease.

In conclusion, this case report series describes three separate presentations of CL that adversely affected the reproductive potential of bucks and a ram. The findings of testicular degeneration described herein are consistent with those reported previously[3] and raise concerns regarding the pathogenesis of CL in the reproductive fitness of small ruminants. Future studies need to focus on the role of phospholipase D exotoxin on both male and female reproduction and its transmission to develop effective vaccines to assist with eradication efforts.[26]

Learning points

- *Corynebacterium pseudotuberculosis* may cause male infertility directly (abscesses) or indirectly (exotoxins).
- Abscess formation associated with CL may present as orchitis, epididymitis, or incidentally.
- Testicular degeneration, either bilateral or unilateral, was reported in all three cases associated with CL.
- All forms of CL-induced reproductive disease may not be detected solely by semen culture.

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Table. Culture results from ejaculate of a Boer buck with infertility (Case 2).

Culture	Isolate #	Isolate	Level
Aerobic	1	*Proteus mirabilis*	TNTC
Aerobic	2	*Staphylococcus aureus*	TNTC
Aerobic	4	*Streptococcus oralis*	TNTC
Aerobic	5	*Corynebacterium sp.*	60
Aerobic	6	*Staphylococcus warneri*	200
Aerobic	3	*Corynebacterium pseudotuberculosis*	TNTC
Anaerobic	51	*Bacteroides fragilis*	TNTC
Anaerobic	55	*Bacteroides pyogenes*	TNTC
Anaerobic	54	*Fusobacterium necrophorum*	TNTC

Figure 1. Asymmetrically enlarged left testicle in a Boer buck associated with CL orchitis (Case 1).

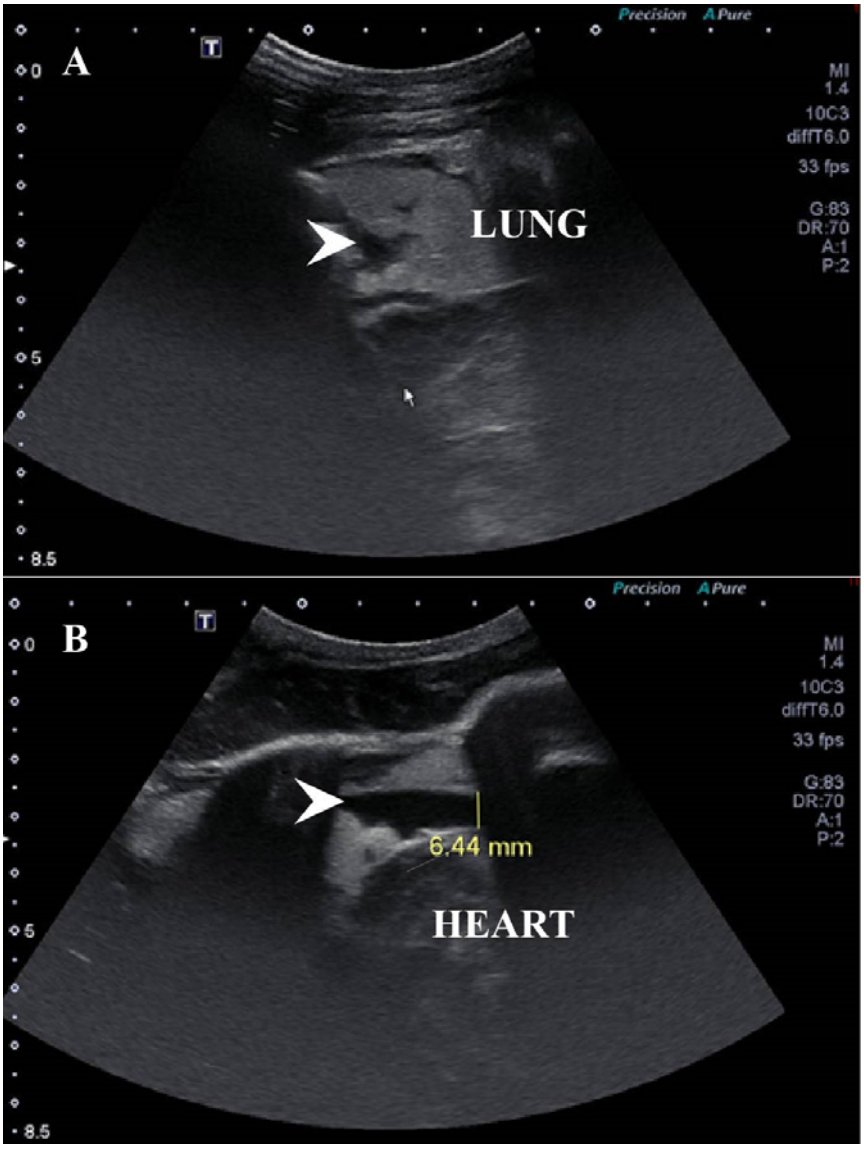

Figure 2. Ultrasonographic images from the thoracic cavity of a Boer buck with thoracic disease presumptively associated with CL (Case 1). Note the pleural (A) and pericardial (B) effusions denoted by the white arrowheads, respectively.

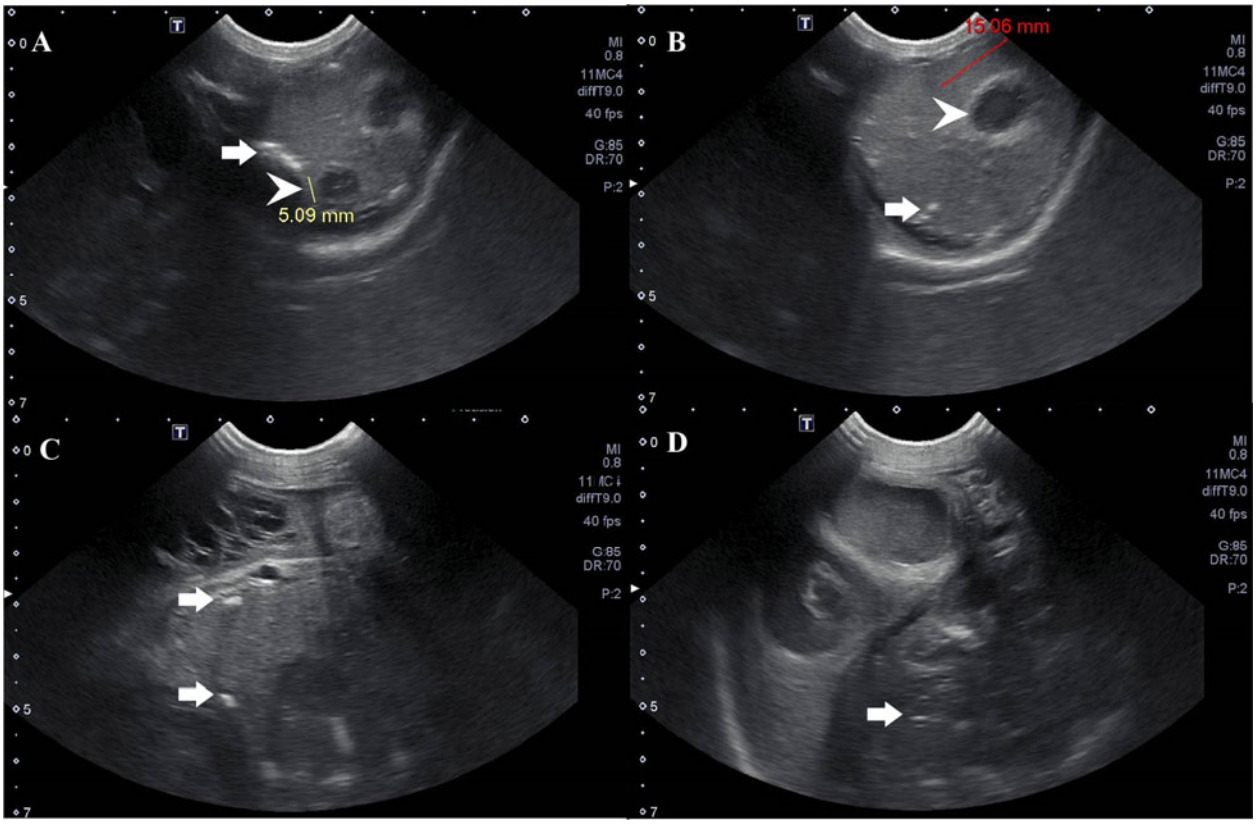

Figure 3. Ultrasonographic images of the right (A, B) and left (C, D) testicles associated with CL orchitis in a Boer buck (Case 1). Large 5- and 15-mm abscesses are apparent in the right testicle (arrowheads). Multiple hyperechoic foci (arrows) are consistent with mineralization and testicular degeneration bilaterally.

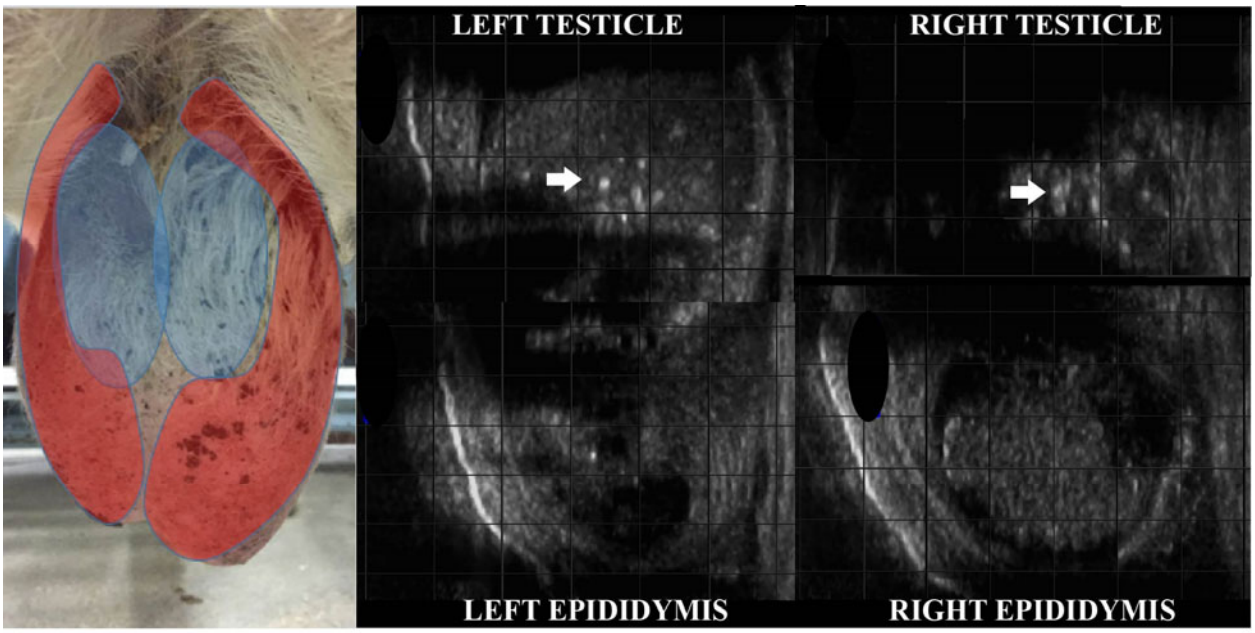

Figure 4. Bilateral epididymitis and testicular degeneration in a Boer buck affected with CL (Case 2). Multiple hyperechoic foci (arrows) were noted within the testicular parenchyma of both testicles and are consistent with mineralization and testicular degeneration. The cauda epididymides were both large, distended, and filled with hyperechoic material.

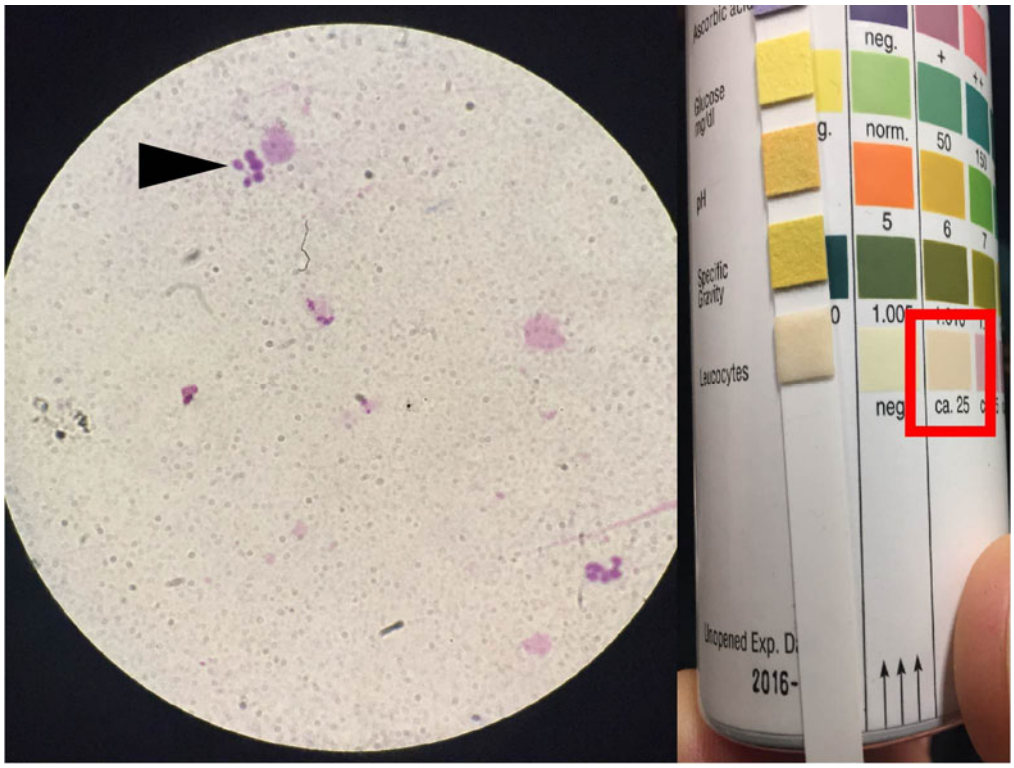

Figure 5. Positive leukocyte esterase test and confirmation of neutrophil presence (arrowhead) and asthenozoospermia in an ejaculate from a Boer buck with bilateral CL epididymitis (Case 2).

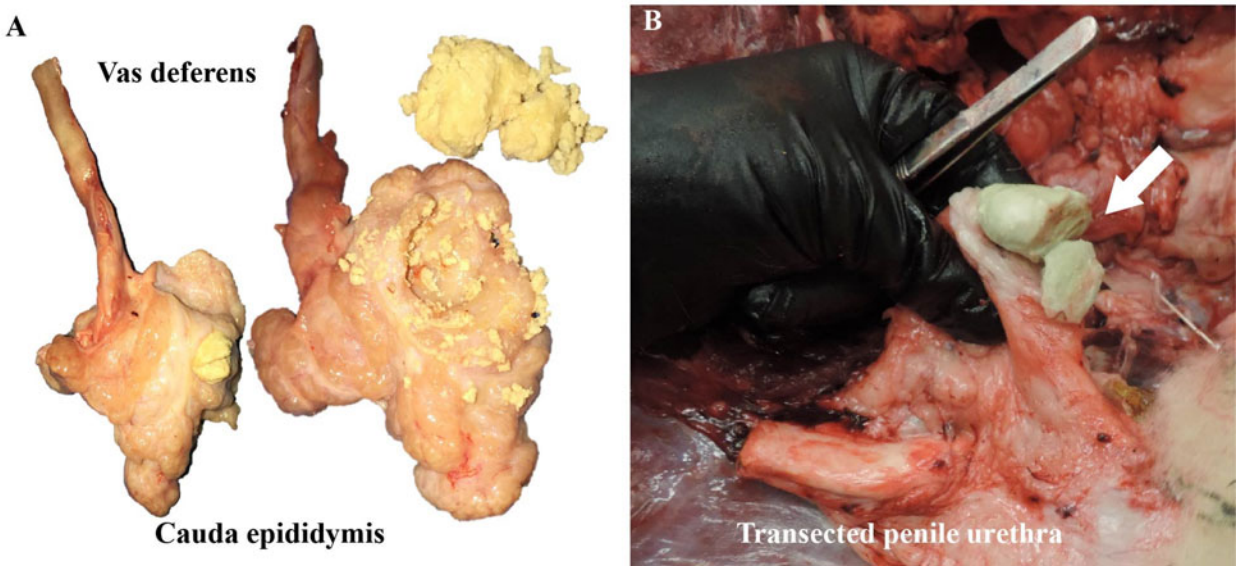

Figure 6. Gross images representing CL abscesses associated with the genitourinary tract in a Montadale ram (Case 3). (A) Image of a caseous peri-epididymal abscess found incidentally during epididymal sperm harvest. Abscess did not communicate with the lumen of the cauda epididymis. (B) Image of a CL abscess (arrow) that is lifted from its location adjacent to the sigmoid flexure of the penile urethra.

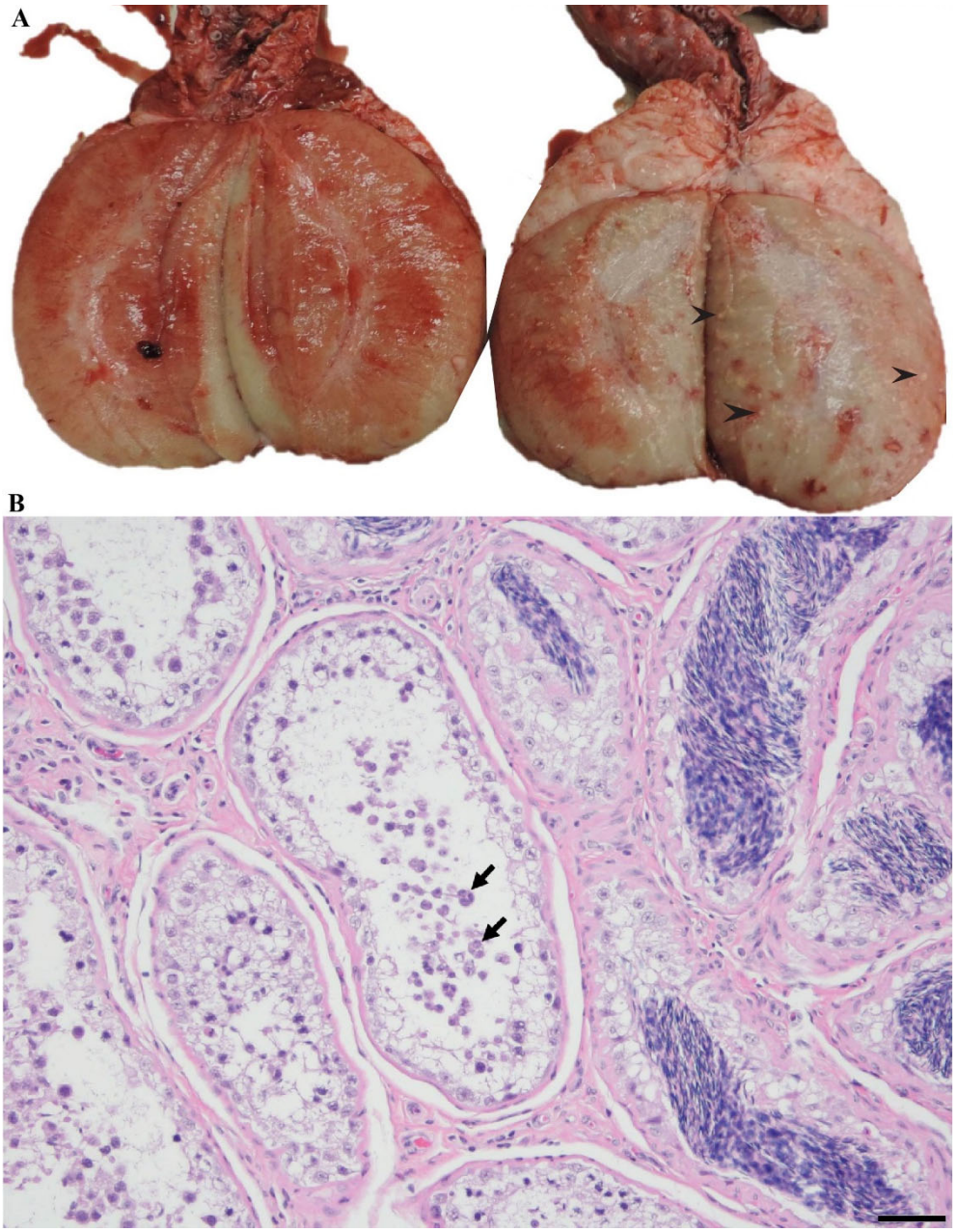

Figure 7. Images representing testicular degeneration associated with CL diagnosis in a Montadale ram (Case 3). (A) Gross image comparing the testicles. The testicle on the right is approximately 2/3 the size of the contralateral testicle and is firm, pale, and contains multiple white flecks (arrowheads) throughout the parenchyma. The cranial aspect of the epididymis is also markedly thickened. (B) Photomicrograph of the smaller testicle from a ram with CL demonstrating testicular degeneration. There is a marked decrease in spermatids and a moderate decrease in spermatogonia within seminiferous tubules that are often lined by vacuolated Sertoli cells and supported by a thickened, hyalinized tubular wall. There are also small numbers of multinucleated (degenerated) spermatogonia within the lumen of seminiferous tubules. Occasional tubules are occluded by dense aggregates of spermatids (spermatozoal stasis). H&E Stain. Bar = 50 um.

(Editor's note: Photographs in this manuscript are available in color in the online edition of Clinical Theriogenology.)