

Update on treatment of vesiculitis in bulls

H. Rovay, A.D. Barth^{*}, M. Chirino-Trejo, M.F. Martínez

Western College of Veterinary Medicine, University of Saskatchewan, Saskatoon, SK S7N 5B4, Canada

Abstract

Four experiments were done to determine: (1) the effectiveness of early detection and treatment of vesiculitis in bulls; (2) whether antibiotic treatment at recommended dosages will result in adequate vesicular gland tissue concentrations of antibiotics to prevent *in vitro* bacterial growth; (3) whether intraglandular injection of antibiotics can be a successful alternative to systemic antibiotic treatment; and (4) the effectiveness of tilmicosin versus tulathromycin for treatment of clinical vesiculitis. In Experiment 1, there was a high rate of spontaneous remission from vesiculitis detected at 9–12 mo of age. Furthermore, there was no advantage for early antibiotic treatment versus no treatment for bulls of this age. In Experiment 2, bacteria on agar plates were exposed to fluid extracted from vesicular gland biopsies after antibiotic treatment of normal, healthy bulls. Although inadequate concentrations of antibiotics were achieved to inhibit bacterial growth when recommended dosages of various antibiotics were administered, doubling the antibiotic dosage increased *in vitro* bacterial growth inhibition. In Experiment 3, relatively nonirritating antibiotics were injected directly into the glands of bulls with clinical vesiculitis, demonstrating that intraglandular injections of antibiotic could be used as a successful alternative to systemic antibiotic treatment. Experiment 4 was a clinical field trial to compare the efficacy of tilmicosin versus tulathromide at recommended dosages for the treatment of clinical vesiculitis. Although the results favored tulathromycin, both antibiotics resulted in clinical cures of vesiculitis.

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1. Introduction

Vesicular adenitis (vesiculitis) was detected in 0.85–10% of yearling bulls during routine breeding soundness evaluations (BSE) [1]. However, there was a much higher incidence of clinical or subclinical vesiculitis (49%) in abattoir-derived specimens [2]. In the experience of the authors (unpublished), as many as 14 of 15 bulls on one farm were affected. A definitive pathogenesis for vesiculitis has not been determined; however, proposed routes of infection include infectious agents ascending

the genito-urinary tract, agents descending from the upper urinary or reproductive tracts, hematogenous invasion, or direct invasion from local sources [3]. A hematogenous source of infection has often been favored as an explanation, since vesiculitis has been associated with high-energy diets [1]. In that regard, high-energy diets predispose to the development of rumenal acidosis, which may lead to rumenitis, followed by bacteremia. *Arcanobacterium pyogenes* and Gram-negative anaerobic bacteria, the most common isolates from liver abscesses of feedlot animals, are also commonly isolated from vesicular gland infections [1,3]. However, bacterial and viral cultures of inflamed vesicular glands were often negative [4]. Congenital abnormalities of the ducts and vessels opening into the urethra at the colliculus seminalis [5], or a lack of synchrony in the ejaculatory

^{*} Corresponding author. Tel.: +1 306 966 7151;
fax: +1 306 966 7159.

E-mail address: albert.barth@usask.ca (A.D. Barth).

process [6], may lead to reflux of semen and urine into the vesicular glands causing inflammation, but not necessarily bacterial infection. Vesiculitis has not been reported in feedlot steers that commonly suffer from ruminal acidosis and liver abscesses. Rapid development of the duct system of vesicular glands during puberty may allow reflux of semen and urine to occur in young bulls; conversely, lack of development of the vesicular glands may spare steers from vesiculitis.

Many types of infectious agents have been isolated from inflamed vesicular glands. Although vesicular gland bacterial isolates are usually sensitive to antibiotics, anecdotal reports indicate a poor success rate for antibiotic treatment, especially with chronic vesiculitis [4]. Surgical removal of infected glands has been the final remedy for some cases. There are no reports that provide data on response to antibiotic treatment for vesiculitis in any age group of bulls. This article describes four experiments to determine the efficacy of antibiotic treatment for vesiculitis.

2. Experiment 1: Early detection and treatment of vesiculitis in bulls [7]

Nine veterinary practitioners examined 2207 bulls of 15 breeds at 17 performance test stations in western Canada. Bulls were examined by transrectal palpation at 9–12 mo of age and assigned to three groups as follows: (1) positive treated group, positive for vesiculitis and receiving subcutaneous injections of 1 mL/30 kg body weight of tilmicosin (Micotil, Provel/Elanco Animal Health, Guelph, ON, Canada), every second day for three treatments; (2) positive control group, positive for vesiculitis and not treated; and (3) negative control group, negative for vesiculitis and not treated. Bulls were considered to be positive for vesiculitis if one or both glands were enlarged and hardened. Transrectal palpation of the glands was done again at a pre-sale evaluation of semen 28–70 d (mean = 42.8 d) after the first examination. Semen was collected by electro-ejaculation and evaluated for the presence of pus and/or leukocytes by light microscopy. Bulls were considered

to have elevated numbers of leukocytes in their semen when the average number of leukocytes was at least one per five microscope fields at a magnification of 1000 $\times$. The prevalence of vesiculitis among breeds was considered for comparison only in groups with >100 animals. The influences of location and veterinarian on the incidence of vesiculitis were analyzed by logistic regression. Proportions of bulls with vesiculitis at the first and second transrectal examinations was compared among groups by Chi-square.

2.1. Results

The proportion of bulls with vesiculitis was 4.4% (97/2207; Table 1). There was no influence of location ($P = 0.35$) or veterinarian ($P = 0.59$) on the prevalence of vesiculitis. After the second evaluation, the total number of bulls with vesiculitis decreased to 1.3% (29/2207); however, seven of these were new cases that developed after the first examination. Only 22 of the 97 original cases remained positive for vesiculitis. Therefore, there was a recovery rate of 75/97 (77.3%) in the original group. At the second examination, there was no difference ($P = 0.99$) in the proportion of bulls with vesiculitis between the positive treated group (15/66, 23%) and the positive untreated group (7/31, 23%).

2.2. Discussion

Vesiculitis was detected as early as 9 mo of age and new cases continued to appear until the end of the experimental period, when vesiculitis was first detected in some bulls at 14 mo of age. Perhaps vesiculitis occurs in bulls at <9 mo of age, but this could not be determined in this study.

The number of bulls with vesiculitis appeared to be much higher in some breeds (e.g., Angus) than in others. However, the numbers of bulls in most of the breed groups were low; therefore, differences in breed prevalence of vesiculitis were likely due to chance rather than a breed effect.

Table 1

The proportion of beef bulls with vesiculitis at 9–12 mo of age that subsequently had vesiculitis (enlarged glands) and the presence of pus or leukocytes in their semen at a second examination, 28–70 d later (mean, 42.8 d)

Group	No.	Enlarged glands at second exam	Leukocytes	Pus
Positive treated	66	15 (21.7%) ^a	10 (14.5%) ^a	4 (5.7%) ^a
Positive untreated	31	7 (21.2%) ^a	2 (6.1%) ^a	2 (6.1%) ^a
Control negative	2110	7 (0.3%) ^b	13 (0.6%) ^b	10 (0.5%) ^b

Within a column, proportions without a common superscript (a and b) differed ($P < 0.01$).

At the time of semen evaluation, 22 bulls from the original group with vesiculitis still had vesiculitis; however, only 12 had leukocytes and only four had pus in their semen. This may support the theory that vesiculitis begins due to reflux of semen and urine into the vesicular glands [6]. Conversely, when inflammation is associated with infection, as infectious organisms are eliminated from the tissue, fewer and fewer leukocytes and gradually less pus would be produced. In that regard, in treated bulls in our previous experiments [8], the numbers of leukocytes and the amount of pus diminished gradually over 2–3 wk after the end of treatment. It appeared that as bacterial infection was reduced, or eliminated by antibiotic treatment and/or natural defense mechanisms, there was a reduction in pus, leukocytes and gland hardness (in that order).

The proportion of bulls with vesiculitis in the positive-control and positive-treated groups was not significantly different at the time of semen collection. Therefore, it appears that under the conditions of this experiment, early detection and antibiotic treatment was not advantageous to reduce the prevalence of vesiculitis at the first semen evaluation. Although early detection and treatment appeared not to be efficacious, the overall incidence of the disease was quite low in this group of bulls. It is noteworthy that the incidence of vesiculitis can be extremely high in some herds [9]. Therefore, early transrectal examination and treatment may be useful in herds with an increased risk for development of the disease.

3. Experiment 2: Effectiveness of antibiotic concentrations in plasma and vesicular gland tissue for *in vitro* bacterial growth inhibition

3.1. Experiment 2a

Twenty-five yearling Angus bulls were randomly assigned to five experimental groups ($n = 5/\text{group}$), to receive one treatment of 20 mL of saline IM (control group), Penicillin G procaine (22,000 IU/kg IM; Pen G Injection, Citadel Animal Health, Cambridge, ON, Canada); Ceftiofur (1 mg/kg IM; Excenel, Pharmacia and Upjohn Sante Animale, Orangeville, ON, Canada); Tilmicosin (10 mg/kg SC, Micotil); or Florfenicol (20 mg/kg IM, Nuflor, Schering-Plough Animal Health, Pointe Claire, PQ, Canada). Blood samples were taken before treatments, whereas blood samples and biopsy samples from the right vesicular gland, were taken between 8 and 12 h after treatment. Plasma and biopsies were stored at -40°C until analyzed.

3.2. Experiment 2b

Eight weeks after the first experiment, the same bulls were randomly re-assigned to five experimental groups ($n = 5/\text{group}$), to receive one treatment of 40 mL of saline IM (control group), the recommended dose of Tulathromycin (2.5 mg/kg IM, Draxxin[®], Pfizer Animal Health, Calgary, AB, Canada), or twice the recommended dose of Penicillin G procaine, Ceftiofur and Florfenicol. Blood samples were taken before treatments and blood samples and biopsy samples from the left vesicular gland were taken between 8 and 12 h after treatment. Plasma and biopsies were stored at -40°C until analyzed.

3.3. Examination of vesicular glands

Vesicular glands were examined by transrectal palpation and ultrasonography before biopsy, on Days 1–5, and at 4 and 8 wk after the biopsy procedure. A crude estimate of vesicular gland volume was determined by length, width and depth measurements through the central region of the glands. A real-time, B-mode scanner (Model SSD-900; Aloka, Tokyo, Japan) equipped with a 7.5 MHz linear-array transducer was used.

3.4. Biopsy procedure

After the rectum was manually evacuated, the pararectal region was clipped and scrubbed with 1% povidone–iodine solution. Local analgesia was achieved by caudal epidural injection of 5 mL of 2% lidocaine HCl (Bimeda-MTC Animal Health Inc., Cambridge, ON, Canada) and by infiltration of the subcutaneous tissue of the ischiorectal fossa with 6 mL of lidocaine. A 1-cm stab incision was made in the skin of the ischiorectal fossa and equine uterine biopsy forceps was pushed through the incision and passed parallel to the rectum toward the vesicular gland. The caudal reflection of the peritoneum was bluntly perforated with the forceps to gain access to the vesicular gland. The vesicular gland was fixed by one hand in the rectum, while the forceps was manipulated with the opposite hand. A single glandular tissue sample (10 mm $\times$ 5 mm) was obtained. No sutures were placed after the procedure was completed. Bulls in the Control group had not been previously treated with an antibiotic; therefore, these bulls were given a single injection of procaine penicillin G at the recommended dose and route after the biopsy was obtained. Bulls in the other groups did not receive any further treatment,

since they had already been treated with their respective antibiotic.

3.5. Bacteriological culture

Isolates of *A. pyogenes* and *Histophilus somni* from clinical cases of vesicular adenitis were used for *in vitro* sensitivity testing. These isolates were known to be sensitive to the antibiotics used in these experiments, with the exception that *H. somni* was resistant to tilmicosin and tulathromycin. Bacterial suspensions were cultured until a turbidity of 0.5 MacFarland standard was reached and then swabbed on blood agar in 94-mm plates. *A. pyogenes* and *H. somni* were inoculated in different plates as described for the standard disk diffusion method [10]. A well was punched in the middle of the agar as a receptacle for plasma or glandular tissue fluid suspensions. Undiluted plasma samples (20 μ L) were directly deposited into agar wells. Biopsies of vesicular gland tissue were trimmed to a standard weight (1 g) and homogenized in 40 μ L of saline; 20 μ L of the resulting suspension was placed in agar wells. As laboratory controls, sterile 6 mm paper discs with antibiotics (Becton, Dickinson and Co, Sparks, MD, USA) were placed on the inoculated agar surface. All procedures were performed under sterile conditions. Plates were incubated at 37 °C with 7% CO₂ for 24 h, and the diameter of zones of bacterial growth inhibition were measured.

3.6. Results

None of the bulls displayed any signs of discomfort at any time after the biopsy procedure. However, in 5 of 25 bulls in Experiment 2a, hematomas were palpable in the pelvic cavity; all resolved spontaneously within approximately 3 wk. In four of the bulls, the hematomas were estimated to be 5–7 cm in diameter and 3 cm deep. In one of the bulls, the hematoma was approximately 20 cm in diameter and 7 cm deep. In Experiment 2b, when the biopsy procedure was repeated in bulls on the opposite gland, none of the bulls developed hematomas. No adverse effects on the biopsied vesicular glands were detected by transrectal palpation or ultrasonography in any of the bulls during Experiments 2a and 2b.

Although bacteria were sensitive to the antibiotics used (as determined by antibiotic disk sensitivity tests), after administration at the recommended dose (Experiment 2a), neither serum nor tissue concentrations of penicillin, ceftiofur, or florfenicol inhibited growth of *H. somni* in any of the bulls. Serum penicillin inhibited growth of *A. pyogenes* in 3 of 5 bulls, and serum

Table 2

Inhibition of *in vitro* growth of *H. somni* or *A. pyogenes* by serum or vesicular gland tissue fluid when samples were collected from beef bulls 6–12 h after treatment with saline (control), or the recommended dose of penicillin, ceftiofur, tilmicosin, or florfenicol ($n = 5$ bulls/group)

Treatment	<i>H. somni</i>		<i>A. pyogenes</i>	
	Serum	Gland	Serum	Gland
Saline	0	0	0	0
Penicillin	0	0	3 (6–8 mm) ^a	0a
Ceftiofur	0	0	0	0a
Tilmicosin ^b	0	0	5 (14–18 mm)	4 (10–17)
Florfenicol	0	0	0	0

^a Diameter of zone of inhibition of bacterial growth.

^b *H. somni* was resistant to tilmicosin.

tilmicosin inhibited growth of *A. pyogenes* in 5 of 5 bulls. Only tilmicosin achieved sufficient concentrations in vesicular gland tissue to inhibit growth of *A. pyogenes* in 4 of 5 bulls (Table 2).

When twice the recommended dose of penicillin, ceftiofur or florfenicol was used, there was an increase in the number of serum and tissue samples that achieved concentrations sufficient to inhibit growth of *H. somni* and *A. pyogenes* (Table 3). Serum penicillin inhibited growth of *H. somni* and *A. pyogenes* in 5 of 5 bulls. Serum ceftiofur inhibited *H. somni* in 5 of 5 and *A. pyogenes* in 0 of 5 cases. Serum florfenicol inhibited *H. somni* in 1/5 and *A. pyogenes* in 1/5 cases. Serum tulathromycin at the recommended dose inhibited *A. pyogenes* in 5/5 cases. Only ceftiofur achieved sufficient tissue concentrations to inhibit *H. somni* (3/5 cases). However, inhibitory tissue concentrations for *A. pyogenes* were achieved in some cases by all antibiotics: 2/5, 1/5, 1/5 and 2/5 cases for penicillin, ceftiofur, florfenicol and tulathromycin, respectively.

3.7. Discussion

The biopsy procedure in Experiment 2a resulted in hematoma formation in some of the bulls, but none of the bulls developed hematomas in Experiment 2b. A possible reason for the difference is that in Experiment 2a, the bulls were younger and their glands were smaller; therefore, a proportionately larger sample was taken from their glands. Secondly, in Experiment 2a, samples were taken from the gland near to the urethra, whereas, in Experiment 2b, biopsy samples were taken from the middle of the gland. Thus, the size of the gland at the time of biopsy and the location of the biopsy might explain the difference in the number of hematomas that developed. In an earlier experiment

Table 3

Inhibition of *in vitro* growth of *H. somni* or *A. pyogenes* by serum or vesicular gland tissue fluid when samples were collected from beef bulls 6–12 h after treatment with saline (control), tulathromycin at the recommended dose, or penicillin, ceftiofur, and florfenicol at twice the recommended dose ($n = 5$ bulls per group)

Treatment	<i>H. somni</i>		<i>A. pyogenes</i>	
	Serum	Gland	Serum	Gland
Saline	0	0	0	0
Penicillin	5 (13–20 mm) ^a	0	5 (17–23 mm)	2 (9,10 mm)
Ceftiofur	5 (21–24)	3 (15–20)	0	1 (15 mm)
Florfenicol	1 (15 mm)	0	1 (15 mm)	1 (12 mm)
Tulathromycin ^b	0	0	5 (8–10 mm)	2 (9,10 mm)

^a Diameter of zone of inhibition of bacterial growth.

^b *H. somni* was resistant to tulathromycin.

(unpublished), 50 vesicular gland biopsies taken from 25 older bulls did not cause any hematomas. Semen samples were collected by electroejaculation from all bulls used in this experiment, approximately 5 wk after the last vesicular gland biopsy was performed. At that time, none of the bulls had any detectable adverse effect of the biopsy procedure. Therefore, the biopsy procedure appeared to be a safe and effective means for obtaining vesicular gland tissue samples from healthy bulls.

The antibiotics administered to the bulls in these experiments resulted in serum or gland concentrations with varying effects on *in vitro* growth of *H. somni* or *A. pyogenes*; however, paper discs containing the antibiotics were effective for growth inhibition of both types of bacteria used in this study (with the notable exception that *H. somni* was resistant to tilmycosin and tulathromycin). The differences in bacterial growth inhibition among plasma and tissue fluid concentrations of antibiotics were likely influenced by concentrations of antibiotic achieved, pharmacokinetic characteristics, and sensitivity of bacteria.

The paper disks for sensitivity testing contained high concentrations of antibiotic and demonstrated only whether high concentrations would inhibit bacterial growth *in vitro*. At 8–12 h after treatment, it was expected that adequate concentrations of the antibiotics used would be present in serum tissues to inhibit bacterial growth if infection was present. However, due to dilution of antibiotics within the blood, inhibition of *in vitro* growth by serum samples should not necessarily be expected.

Based on Experiment 2a, recommended dosages of penicillin, ceftiofur and florfenicol may not achieve sufficient concentrations in vesicular gland tissue to inhibit growth of the strains of *H. somni* and *A. pyogenes* used in these experiments. The *H. somni* strain used in this experiment was resistant to

tilmycosin, therefore, tilmycosin did not inhibit *in vitro* growth of *H. somni*, but concentrations were sufficient to inhibit *A. pyogenes* in 4 of 5 cases. Therefore, tilmycosin might be an antibiotic of choice for treatment of vesiculitis involving these bacteria.

In Experiment 2b, using twice the recommended dosage of antibiotics improved the chance of bacterial growth inhibition in vesicular gland tissue for all antibiotics tested. Perhaps treatment failures in clinical cases of vesicular adenitis are due to inadequate concentrations of antibiotics developing within the gland tissue. Field trials are needed to study the treatment responses of clinical cases of vesiculitis to the recommended dose of various antibiotics, as well as the use of these antibiotics at doses exceeding label recommendations.

4. Experiment 3: Intraglandular injection of antibiotics for the treatment of vesicular adenitis [8]

In an earlier experiment, intraglandular injection in normal healthy bulls of 10% of the daily recommended parental dose of procaine penicillin G (22,000 IU/kg IM), or ceftiofur (1 mg/kg kg IM), dissolved in saline (final volume, 6 mL), caused minimal tissue reaction without any prolonged ill effect to the vesicular glands. The method of intraglandular injection of antibiotics was as follows. Preparation of the region of the pararectal fossa was similar to that for vesicular gland biopsy. After stab incision was made in the skin of the ischiorectal fossa, a custom-made, blunt, 4-mm o.d. needle guide was pushed through the incision and passed pararectally up to the vesicular gland. The caudal reflection of the peritoneum was bluntly perforated with a short jab. The vesicular gland was fixed by one hand in the rectum, while the needle guide was manipulated with the opposite hand. A 44-cm long 18-gauge needle

containing antibiotic (to avoid injecting air into the gland) and connected to a syringe with antibiotic was directed through the needle guide into the gland. The antibiotic was slowly injected into the gland.

Fourteen untreated beef bulls with clinical vesicular adenitis were used in this experiment. Eight bulls had unilateral vesiculitis (left, $n = 3$; right, $n = 5$) and six bulls had bilateral vesiculitis. Since the bull owners were unwilling to leave their bulls untreated, a period of 3 wk from the time of diagnosis in the field until experimental treatments were commenced was considered to be a control period for these bulls. The 14 bulls were then treated by an intraglandular injection of an antibiotic.

Semen for bacterial culture was collected by electroejaculation at the initial evaluation. After penile protrusion, the glans penis was grasped with a sterile surgical sponge and the tip of the penis was swabbed with betadine and dried with a sterile sponge. A spurt of semen was directed into a sterile vial held horizontally near the penis. Semen was swabbed on sheep blood agar and MacConkey agar. All plates were incubated at 37 °C in an atmosphere of 7% CO₂. Biochemical characterization and antimicrobial sensitivity tests for bacteria isolated were performed following the National Committee for Clinical Laboratory Standards M31-A recommendations [10].

Bulls were subjected to transrectal palpation and ultrasonography of the vesicular glands and semen collection by electroejaculation before treatment and every 4 d (11 times) after treatment.

Ceftiofur was injected into affected glands as the initial treatment in 13 of the 14 bulls; in the absence of evidence of recovery within 3 wk, a second intraglandular treatment with penicillin ($n = 6$) was done. One bull was treated only once with an initial injection of penicillin. Three bulls that did not recover from vesiculitis after two intraglandular antibiotic injections (ceftiofur and penicillin) received three treatments of tilmicosin subcutaneously (Micotil, 10 mg/kg) at the recommended dose, 48 h apart.

Recovery from vesiculitis was deemed to have occurred when two or more consecutive semen samples were free of blood and pus, and semen smears had less than one leukocyte per five microscope fields at 1000× magnification. The presence of blood, pus and leukocytes in the semen, and vesicular gland hardness were scored from 0 to 4. For blood: 0: absence, 1: slight tinge, 2: pink, 3: red, and 4: dark red ± clots. For pus: 0: absence, 1: a few 1 mm diameter flakes, 2: a few 2 mm diameter curds, at the bottom of the semen column after a settling period of 5 min, 3: ≤0.5 mL, and 4: >0.5 mL

of pus at the bottom of the semen column immediately after semen collection. For detection of leukocytes, semen was allowed to settle for 5 min, and then semen was sampled from the bottom of the semen column for preparation of an eosin–nigrosin stained semen smear. The leukocyte score was: 0: absence, 1: <2 leukocytes per 1000× field on microscopy, 2: 2–5 leukocytes per field, 3: 5–10 leukocytes per field, and 4: >10 leukocytes per field. The hardness of the vesicular glands was subjectively scored by transrectal palpation as 1: consistency of a normal gland, 2: slightly increased firmness, 3: moderately hard, and 4: hard.

4.1. Ultrasound imaging

Transrectal ultrasonographic examinations were performed immediately before, immediately after, and at 24, 72 and 168 h after intraglandular antibiotic treatment to monitor changes in glandular echotexture and changes in volume. A crude estimate of volume was determined by length × width × depth measurements through the central region of the glands. A real-time, B-mode scanner (Model SSD-900; Aloka, Tokyo, Japan) equipped with a 7.5 MHz linear-array transducer was used. The ultrasound scanner had a built-in program that allowed for the construction of a histogram that indicated the intensity distribution of image pixels within a randomly selected area (1 cm²). The data provided by the built-in program included:

<i>T</i>	the total number of pixels in the specified area
<i>L</i>	the intensity level that occurred most frequently in the specified area
<i>M</i>	the number of pixels corresponding to the intensity level that most frequently occurred in the specified area
<i>MN</i>	the mean intensity level in the specified area
<i>S.D.</i>	the standard deviation of the intensity distribution in the specified area

A mean high intensity pixel value was also calculated based on *L* multiplied by its frequency *M*. Percentage of *MN* and *S.D.* were also analyzed using the ultrasound scan at Time 0 (before treatment) as the reference control equal to 100%.

4.2. Statistical analysis

Pearson's correlation coefficients were calculated for semen characteristics (density, gross motility, individual motility, percentage of normal spermatozoa, presence of blood, pus, or leukocytes). Scores of consistency of the vesicular glands and the presence of

blood, pus or leukocytes among semen samples were compared by the Kruskal–Wallis non-parametric test. Mean ranks between groups were further compared by the Mann–Whitney test. The MN and S.D. of pixel distribution were analyzed by analysis of variance in a 2×2 factorial design (Status: affected or healthy gland; Time: before and 7 d after treatment). Data and graph processing were performed with Microsoft Office Excel and statistical analyses were conducted with SPSS for Windows (SPSS v. 11.5, SPSS Inc. 2002, USA).

4.3. Results

A. pyogenes was the most common bacterium isolated from the infected bulls. In addition, there were isolates of *Acinetobacter* sp., *Streptococcus* sp., *Pasteurella* sp. and *Corynebacterium* sp. All bacteria isolated from the different bulls were sensitive to ceftiofur, penicillin, and tilmicosin. Seven of 13 bulls recovered after one intraglandular injection of ceftiofur, and 1 of 1 bull recovered after one intraglandular injection of penicillin. Three of 6 bulls that did not recover after one injection of ceftiofur recovered after a second intraglandular injection with penicillin. Three bulls that did not recover after a ceftiofur and a penicillin intraglandular treatment, recovered after receiving three treatments of tilmicosin by subcutaneous injection.

There was a difference ($P < 0.05$) among successive ejaculates in pus, leukocytes, and blood (Fig. 1). Ejaculates 8–11 had less pus than the first two ejaculates ($P < 0.05$), and less leukocytes than the first five ejaculates ($P < 0.05$). Ejaculates 6–11 had less blood than Ejaculates 2 and 3 ($P < 0.05$).

The mean change in consistency score of the glands (transrectal palpation) remained unremarkable from the first to the last examination in most bulls ($P = 0.63$); however, in three bulls, the consistency score decreased from 4 to 1 by the time of the last examination. When inflamed and healthy vesicular glands were compared by ultrasonography before and 1 wk after treatment, there was no effect of gland health status, time, or health status by time interaction on the MN ($P = 0.3$, $P = 0.46$, and $P = 0.64$, respectively) or S.D. ($P = 0.16$, 0.77 , and 0.20 , respectively) of pixel distribution.

4.4. Discussion

In this experiment, intraglandular injection of ceftiofur or penicillin in beef bulls via the ischiorectal fossa appeared to be safe and effective in the treatment of vesicular adenitis. A major fault of this experiment

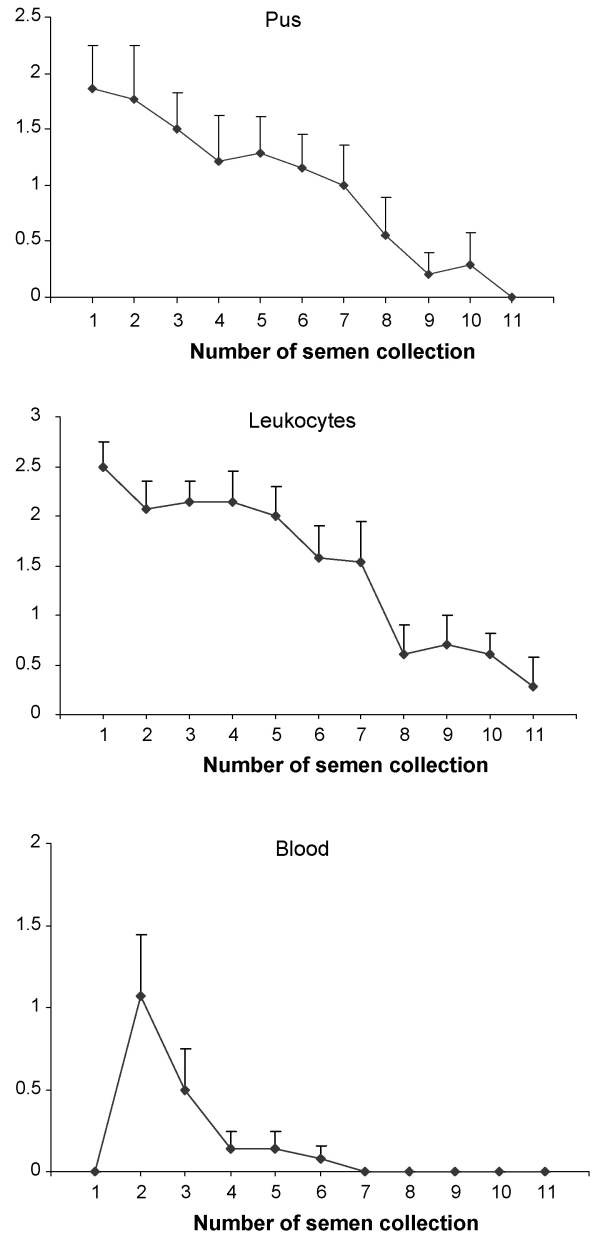


Fig. 1. Mean ($\pm$ S.D.) scores representing the presence of pus, leukocytes and blood in the semen samples obtained from all bulls affected with vesicular adenitis.

was a lack of a contemporary control group, in which bulls with vesiculitis were not treated to ensure that any recoveries due to intraglandular antibiotic treatment was not due to spontaneous recovery. To compensate for a contemporary control group, a control period of at least 3 wk was allowed before bulls were treated; none of the bulls recovered during this time period.

Ultrasonography can be used to evaluate the characteristics of the glands prior to and after

intraglandular antibiotic treatment. Evaluation of vesicular glands by transrectal palpation was also useful in monitoring the effect of treatments, but semen collection is likely the most useful method to evaluate the progress of recovery from vesiculitis.

5. Experiment 4: Treatment of field cases of vesicular adenitis with tilmicosin and tulathromycin

Two antibiotics, tilmicosin and tulathromycin, were chosen for use in a field test for efficacy in the treatment of clinical vesiculitis. Tilmicosin and tulathromycin were chosen over other antibiotics, based on previous experimental results of vesicular gland tissue fluid inhibition of *in vitro* bacterial growth. In addition, there was evidence that tilmicosin and tulathromycin accumulated in macrophages and neutrophils and were subsequently released slowly from such cell types [11]. Thus, immune defense cells would carry these antibiotics into sites of infection [12]. Antibiotic coverage was for 6 d, i.e., a single tulathromycin treatment, or two tilmicosin treatments 72 h apart, at a dose indicated by the label information.

A mail-out of an experimental protocol was done to veterinarians in large animal practices in Alberta, Manitoba, and Saskatchewan, Canada. A total of 180 veterinary practitioners were contacted and offered financial incentive to participate.

Eligible cases of vesiculitis were considered to be those in which clots or flakes of pus were visible in semen samples, white blood cells were found at ≥ 1 per five microscope fields at 1000 $\times$ magnification, the vesicular glands were enlarged and hardened, and there was no record of previous antibiotic treatment.

Response to treatment was determined 21–28 d after the beginning of treatments. A positive response was considered to be: no clots of pus in the semen, no flakes of pus in the bottom of the tube after 5 min of settling time, and ≤ 1 white blood cells per microscope field at 1000 $\times$ magnification.

Gland size and hardness were often reduced in bulls with a positive response, but this was not required to qualify as a positive response. Participating veterinarians were required to obtain a sample of semen by sterile means, submit it to a provincial laboratory, and report the results. Semen collection for bacterial culture was done after penile protrusion by electroejaculation. The glans penis was grasped with a sterile surgical sponge and the tip of the penis was swabbed with betadine and dried with a sterile sponge. A spurt of semen was directed into a sterile vial held horizontally

near the penis. In the control group, the possibility of spontaneous remission was investigated at >3 wk from the discovery of the case.

5.1. Results

Seventeen veterinarians participated in the study, involving 65 bulls. The recovery rate was higher for bulls treated with Tulathromycin (22/25 = 88%) than for Tilmicosin (11/23 = 48%) and both antibiotics resulted in a higher recovery rates than occurred the untreated control group (0/17; $P < 0.01$). In the tulathromycin group, six bulls were mature (four 2 y olds and two 3 y olds), but the three bulls that did not recover were yearlings. In the tilmicosin group, five bulls were mature (three 2 y olds, one 4 y old, and one 6 y old). Two of the 2-y-old bulls treated with tilmicosin recovered, but the other mature bulls did not. In the control group, all bulls were yearlings and none recovered.

5.2. Discussion

Based on the outcome, both Tilmicosin and Tulathromycin should be useful in the treatment of vesiculitis. Studies of vesicular gland tissue concentrations of antibiotics indicated that tilmicosin concentrations achieved may be inadequate in some bulls and therefore, an increased dosage, or a more frequent treatment regime than that recommended on the label, might give better results. Anecdotal reports of treatment of clinical cases in the field appeared to confirm this finding.

Many of the semen culture sensitivity tests indicated that the organisms isolated were resistant to tulathromycin and tilmicosin. This included organisms considered likely to be pathogens; however, a large proportion of isolated organisms were considered contaminants. The organisms considered pathogens were *H. somni* and *A. pyogenes*, and the main contaminants were *Pseudomonas* spp., *Proteus* spp., *E. coli*, *Staphylococcus* spp. and *Streptococcus* spp. Nevertheless, recovery occurred after treatment in a large proportion of the cases.

Both antibiotics resulted in cures of vesiculitis (as defined for this study), including some bulls that were ≥ 2 y old. Although the time of onset of vesiculitis in these bulls could not be determined, it is likely that the infections were of a chronic nature. If this is so, then a 6-d course of antibiotics was apparently able to overcome chronic infections in some bulls. Further studies are needed to determine the relapse rate in bulls that appear to recover after antibiotic treatment.

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