Reticulo-ruminal milk accumulation (ruminal drinking) in five pre-ruminant white-tailed deer (*Odocoileus virginianus*) in Texas

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Abstract

Ruminal drinking is a condition that has been extensively reported in pre-ruminant calves, and is characterized by an insufficient esophageal groove reflex that causes ingested milk to spill into the reticulum and/or rumen instead of entering the abomasum directly. In calves, milk that spills into the rumen undergoes rapid microbial fermentation, producing lactic acid and other volatile fatty acids, and lowering the ruminal pH. This reduced ruminal pH predisposes the animals to ulceration and necrosis of the rumen mucosa, as well as maldigestion and recurrent tympany. We investigated five cases of ulcerative rumenitis in white-tailed deer (WTD: *Odocoileus virginianus*) fawns from 2009 to 2014. Four of these fawns were females with ages ranging from 2 to 30 days. Clinical findings in the fawns included poor body condition, severe diarrhea, and sudden death. Gross changes consistently observed included large amount of milk and milk curds in the rumen, reticulum and occasionally omasum, white-colored and bloody diarrhea, severe muscle wasting with extensive loss of visceral fat. On microscopic examination, the most consistent finding among three of the animals was multifocal necrourculcerative rumenitis admixed with basophilic globular material (milk), while the other two animals had unremarkable histopathologic findings. Aerobic and anaerobic bacterial cultures of samples collected revealed mixed bacterial growth suggestive of secondary opportunistic invasion. Molecular diagnostics ruled out the possibility of Bluetongue and Epizootic Hemorrhagic viral infections. This report describes for the first time, clinical, gross, and microscopic changes associated with reticulo-ruminal milk accumulation in WTD fawns across farms in Texas, and complements those of earlier studies in calves.

Key words: pathology, reticular groove reflex, rumen, ruminal drinking, white-tailed deer.

Introduction

White-tailed deer (WTD), *Odocoileus virginianus*, is one of the most common wildlife species in the United States (US). It is also present in Canada, Mexico and some areas of South and Central America (International Union for Conservation of Nature http://www.iucnredlist.org). Highly valued as a major game species, WTD are farmed in the US for both venison and hunting. In recent years, rapidly increasing hunting demand has led to a substantial growth of the industry with over $3 billion revenue/year (27). This increased demand has made the practice of deer farming common, with the implementation of new artificial methods to raise newborns in a more efficient and economically viable approach. These new practices may predispose newborns to impaired health conditions commonly reported in other domestic animal species but not wildlife ruminants such as WTD.
Fawns rely entirely on the doe for nursing and grooming especially in the first few days of life (12, 29). Intensive deer farming practices are usually associated with weaning within the first 24 hours post-partum, and fawns are subsequently bottle and/or bucket-fed with milk or milk replacer. Weaning is a stressful situation for the fawn and can predispose to a number of conditions that negatively impact the animal’s health. Young ruminants are unique at birth in terms of their gastrointestinal system. At birth, the rudimentary state of reticulo-rumen and omasum forces neonatal ruminants to function as monogastric animals, utilizing only the abomasum to prepare the ingested milk or milk-based diets for further digestion in the small intestine. Transition from monogastric to a ruminant animal requires adequate size and development of the reticulo-rumen for efficient utilization of dry and forage-based diets (29, 30). In WTD, usually at 20 days of age, the forestomachs are capable of digesting small amounts of forage (12, 30). However, complete development of the forestomachs occurs between four to five months of age under natural conditions (30). The esophageal groove (also called reticular or gastric groove) is a muscular structure that begins at the cardia and extends to the reticulo-omasal opening. This groove forms a tube-like structure that directs ingested milk into the abomasum, bypassing the forestomachs (Fig. 1a). It is characterized by folds of smooth muscle that close in response to vagal nerve stimulation initiated by the contact of sodium chloride and sodium bicarbonate in milk with the sensory nerves of the oral cavity and pharyngeal area (25). The esophageal groove can also close upon visual, auditory and olfactory stimulation relating to milk consumption (23). An inappropriate sucking reflex by the calf causes milk to be “gulped” instead of “sucked” as it happens when milk-replacer is fed in buckets, causing incomplete esophageal groove closure, predisposing calves to diarrhea secondary to malabsorption (25, 27). The digestive enzymes, esterase and pancreatic lipase within the saliva of the neonatal ruminant, help to facilitate digestion of fats within the milk curds in the abomasum (28). Further digestion and absorption of nutrients takes place in the small intestine. However, the only carbohydrate that neonatal ruminants can digest and utilize is lactose because of an increased production of lactase by the small intestine shortly after birth (25, 26). Other enzymes such as maltase and sucrase are absent at this stage (below 30 days of age), but are produced in large quantities with an increase in age as intestinal lactase drops (9, 24).

In young ruminants, a dysfunction of the esophageal groove reflex causes ingested milk to spill into the reticulum and/or rumen, and because neither the rumen nor the reticulum are physiologically functional, milk that enters these undeveloped stomachs usually undergo fermentation, producing digestive disturbances, a condition usually referred to as ruminal drinking (RD). Most studies investigating RD syndrome have focused on calves with very limited studies about RD in other ruminant species such as the WTD. RD in milk-fed calves is a well-recognized syndrome that is most commonly observed in two to eight-week-old milk-fed calves (10). In the rumen, a carbohydrate fraction of the ingested milk undergoes bacterial fermentation with the production of acetic, butyric and lactic acid (D and L isomer), with subsequent fall in the ruminal pH to about 5 and below (24). This low pH (ruminal acidosis) can result in ulceration of the ruminal mucosa with subsequent development of hyperkeratosis and parakeratosis overtime that can lead to impairment of ruminal motility and recurrent tympanism (3). Other secondary changes associated with RD in other organs such as the small intestine include villous atrophy and reduced lactase activity of the brush border epithelium of the villi that also can lead to maldigestion and malabsorption (4, 10).

Two different forms of fermentative ruminal acidosis in calves have been described, an acute and a chronic form. In the acute form, dysfunction of the esophageal groove is usually superimposed on another pre-existent condition (13). This acute, and usually severe form, is typical in young calves fed cow’s milk in buckets during the first few weeks of life. In the chronic form, dysfunction of the esophageal groove occurs as a result of stressful condition such as transportation, dehorning and weakness from perinatal maladjustment syndrome (31). Neonatal diarrhea has also been implicated as a cause of esophageal groove dysfunction in calves, predisposing them to RD syndrome (23).

RD has only been reported in calves and artificially fed lambs, but never in other ruminant species, including WTD. This report describes for the first time, the clinical, gross and histopathological characteristics associated with reticulo-ruminal milk accumulation in five WTD fawns.

Material and methods

Clinical history and presenting signs

From May 2009 to June 2014, a total of five WTD fawns (one male and four females) ranging from 2 to 30 days old were presented to the Texas A&M Veterinary Medical Diagnostic Laboratory (TVMDL). Whole bodies were submitted for necropsy in four of the cases (cases 1, 2,3 and 5), and for the fifth case (case 4), fresh and formalin-fixed heart, lung, liver, kidney; fresh spleen, ruminal content and intestinal culture were submitted for evaluation. The fawns were from distinct farm locations across Texas. Clinical information was obtained from accompanying necropsy submission forms. These WTD fawns presented with sudden death (cases 1, 2, and 4), inappetence, severe diarrhea and cachexia (cases 3 and 5). In addition, case 4 was reported to be pen-raised and on milk replacement.
Gross and histopathologic examinations

Gross examination was performed, and tissue samples were collected for histopathology and bacteriology. For histopathologic analysis, tissue samples were fixed in 10% neutral buffered formalin, processed routinely, and embedded in paraffin wax. Sections 5μm thick were prepared from formalin-fixed, paraffin-embedded liver, spleen, kidney, heart, lung (cases 1, 2, 3, 4 and 5) rumen, reticulum, intestine, brain and skin samples (cases 1, 2, 3 and 5). Sections were thereafter stained with haematoxylin and eosin (HE).

Bacterial culture

Sections of fresh rumen and small intestine collected during necropsy were submitted for anaerobic and aerobic bacterial culture in all cases, except case 1.

Molecular diagnostics

Spleen samples from two animals (cases 4 and 5) were analyzed for Epizootic Hemorrhagic Diarrhea virus (EHD) and Bluetongue virus using PCR assays. Methodologies for the PCR assays were based on previously published studies (20).

Results

Clinical presentation

Table 1 below shows a complete representation of the cases. Three of the five fawns (cases 1, 2 and 4) died acutely, cases 2 and 5 presented with white colored and blood stained diarrhea, and cases 1, 2, 3 and 5 had poor body condition (cachexia).

Gross findings

During gross examination, all animals had moderate to large amounts of fresh and coagulated milk in the rumen (Fig. 1b). In two cases (40%), the reticulum and omasum were also involved (cases 1 and 5). Case 3 had multifocal, pinpoint to 2cm eroded areas on the rumen mucosa that deeply penetrated into the underlying submucosa (ulcers) (Fig. 1c). In two cases (cases 2 and 3), the mucosa of the small and large intestine was dull with multifocal to coalescing dark red areas. Gross findings in other organs were unremarkable.

Microscopic findings

Despite the gross presence of milk in all cases, only three fawns had microscopic evidence of necroulcerative rumenitis admixed with a basophilic globular material (milk) (cases 1, 2 and 3). In these cases, the ruminal mucosa was multifocally ulcerated with the underlying submucosa expanded by edema and variable degrees of inflammatory cells, including viable and degenerated neutrophils, macrophages, and lesser numbers of lymphocytes and plasma cells (Fig. 1d). Adjacent to the necrotic areas, there was evidence of granulation tissue with large numbers of reactive fibroblasts (case 3). Four of the five cases (cases 1, 2, 3 and 5) had small to large numbers of bacterial colonies (filamentous and cocci). In case 2, the mucosa of the small intestine also had moderate amounts of necrotic debris. Two animals had minor large intestinal mucosal changes, characterized by small number of neutrophils admixed with necrotic debris and a mixed population of bacterial colonies (cases 2, 3). There were no significant microscopic findings in all other organs examined (reticulum, liver, spleen, kidney, heart, lung, brain and skin).

Bacteriology

Aerobic and anaerobic bacterial culture revealed mixed bacterial growth (Escherichia coli, Proteus, Enterococcus, Bacillus sp., Pseudomonas aerugiosa, Arcanobacterium pyogenes), interpreted as secondary opportunistic bacterial invasion.

Molecular diagnostics

The two animals (cases 4 and 5) were negative for both EHD virus and Bluetongue virus using PCR assays.

Discussion

This current report describes for the first time in pre-ruminant white-tailed deer (WTD), clinical, gross and microscopic changes associated with reticulo-ruminal milk accumulation (ruminal drinking).

Ruminal drinking syndrome (RD) is a well-characterized condition in calves and lambs (3, 8, 10, 18, 22, 31, 32, 34). However, it has never been reported in wildlife ruminant species. It may be expected that since WTD are ruminant species, the pathogenesis and degree of manifestation of RD syndrome should be similar to that of calves, but currently there are no studies to confirm or refute this statement. However, one known difference between calves and WTD fawns in relation to the development and functionality of the forestomachs, is age (17, 29, 30). This age difference may not only have an impact on the time of occurrence of RD, but also the degree of manifestation of the condition. Notably, the development of the rumen into a completely functional organ is slower in deer fawns compared to calves (30).
Table 1. Complete representation of the clinical signs and pathological findings of the five cases of white-tailed deer (WTD) fawns with reticulo-ruminal milk accumulation (ruminal drinking). The ancillary tests (aerobic and anaerobic bacterial culture) performed on four of the cases are also shown.

<table>
<thead>
<tr>
<th>Case number</th>
<th>Sex</th>
<th>Age (days)</th>
<th>Clinical signs</th>
<th>Gross findings</th>
<th>Histological findings</th>
<th>Ancillary tests</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Male</td>
<td>2</td>
<td>Sudden death</td>
<td>Fresh and coagulated milk in the rumen, reticulum and omasum.</td>
<td>Necrotizing rumenitis with ulceration, filamentous bacteria and milk.</td>
<td>N/A</td>
</tr>
<tr>
<td>2</td>
<td>Female</td>
<td>2-3</td>
<td>Diarrhea, Sudden death</td>
<td>Fresh and coagulated milk in the rumen.</td>
<td>Multifocal mild ulcerative rumenitis; Moderate amount of milk on the mucosal surface of the rumen, admixed with of large numbers of bacterial rods.</td>
<td>Bacteriology (aerobic and anaerobic cultures- <em>E. coli</em>, <em>Enterococcus</em> sp., <em>Proteus</em> sp., <em>Bacillus</em> sp.).</td>
</tr>
<tr>
<td>3</td>
<td>Female</td>
<td>30</td>
<td>Cachexia, Weakness</td>
<td>Fresh and coagulated milk in the rumen.</td>
<td>Severe transmural necroulcerative rumenitis with intralesional deposition of milk admixed with the presence of bacterial colonies and granulation tissue.</td>
<td>Bacteriology (aerobic and anaerobic cultures- <em>Arcanobacterium pyogenes</em>, <em>Pseudomonas aeruginosa</em>).</td>
</tr>
<tr>
<td>5</td>
<td>Female</td>
<td>7-10</td>
<td>White-colored and blood stained diarrhea, Lethargy</td>
<td>Fresh and coagulated milk in the rumen, reticulum and omasum.</td>
<td>Large amounts of bacteria in the rumen.</td>
<td>Bacteriology (aerobic and anaerobic cultures- <em>Escherichia coli</em>, mixed bacterial growth).</td>
</tr>
</tbody>
</table>

RD syndrome has been extensively reported in calves less than four weeks of age (14, 18, 34), consistent with this, the fawns in these case series range from zero to four weeks of age. Additionally, in calves, transportation stress, neonatal maladjustment syndrome, weakness, neonatal diarrhea and subsequent malabsorption are commonly reported underlying conditions predisposing to RD (14). We therefore suggest that the diarrhea reported in cases 2 and 5 may have led to RD syndrome observed in this fawn. Moreover, it was not determined if these fawns were artificially fed, and no apparent cause of ruminal drinking was identified during clinical, gross and histopathological examinations. Only case 3 was reported to be weak before death, the other fawns (cases 1, 2, and 4) died acutely and there was no history of weakness or diarrhea. Bucket-fed calves have been shown to be prone to RD (1). However, only case 4 was reported to be bucket-fed. Consequently, we suggest that RD in this fawn could be due to this type of feeding procedure. Nevertheless, an important issue for future investigation is whether artificial feeding (bucket or bottle-feeding) in WTD is significant.

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Ruminal acidosis resulting from RD (observed in these fawns) must not be confused with the traditional ruminal acidosis observed in older animals, which is associated with grain overload and subacute ruminal acidosis. The former occurs in young ruminants that survive entirely on milk diets while the latter occurs in adult ruminants that ingest dry feeds and forages (15). In grain overload and subacute ruminal acidosis, animals are exposed to large quantities of highly fermentable carbohydrates, which modifies ruminal microbiota leading to increased production of D and L isomers of lactic acid and other volatile fatty acids. The resulting low pH (5.5 and below) causes ulceration and necrosis of the ruminal mucosa. These conditions usually subsides as the intake of highly fermentable carbohydrates is reduced (15).

In contrast, ruminal acidosis in RD neonates results from fermentation of the ingested milk in the rumen leading to the production of D and L isomers of lactic acid and other volatile fatty acids that can lead to low ruminal pH, systemic metabolic acidosis (elevated serum level of D-lactate), a high anionic gap, and eventual ulceration and necrosis of the ruminal mucosa (7, 16, 25, 26).

Absorption of microbial end products (volatile fatty acids such as butyrate and propionates) from ingested dry feeds aid the development and maturation of the ruminal epithelium in terms of papillae growth, size and function (15). Therefore, in young ruminants that survive entirely on milk or milk-based diets, the development of the rumen is reduced with little or no ruminal epithelial activity and volatile fatty acids absorption. Nevertheless, rumen size increases proportionately with the body size as...
the animal grows regardless of ruminal epithelial development and maturation (33).

The absorption of D-lactate into tissues occurs via proton-dependent monocarboxylate transporter (MCT). In excess bacterial fermentation, there is a high concentration of protons that enhances absorption of D-lactate leading to metabolic acidosis (11, 35). Previous studies have suggested that absorption of D-lactate into brain tissue via the proton-dependent MCT expressed in brain tissue is a cause of depression and weakness amongst other signs (11, 14, 22, 28). It can thus be suggested that the depression and weakness observed in cases 3 and 5 may be a result of D-lactate absorption. Unfortunately, in all the cases in this report, D-lactate level as well as pH levels of the ruminal fluids were not measured, but upon opening the rumen during gross examination, a foul acidic smell was perceived consistent with ruminal acidosis. Measuring the serum D-lactate level and pH of ruminal fluid from these fawns would have corroborated the susceptibility to rumen mucosa ulceration.

Clinical signs presented in these fawns included anorexia, severe starvation (cases 1, 2, 3 and 5), reduced absorption and digestibility, diarrhea with clay-like feces (cases 2 and 5), which are consistent with those observed in calves with RD (8). Previous studies have associated RD with sudden death resulting from severe nutritional imbalance, systemic metabolic acidosis secondary to D-lactate uptake via the proton-dependent MCT, or by invasion and proliferation of opportunistic bacterial or fungal agents due to alteration in microbiome balance (25).

In agreement with this, the fawns in these case series were either found dead (cases 1, 2, and 4) or died within few days of presenting clinical signs.

Microscopic analysis revealed concomitant bacterial colonies in four of the five cases (cases 1, 2, 3 and 5). We suggest that the presence of milk in the rumen of these fawns (Fig. 1b) altered the ruminal microbiota leading to abnormal proliferation of resident bacteria. Histopathological findings in calves with chronic RD reveal the presence of milk in the rumen with varying degrees of ulcerative rumenitis (3). The ruminal epithelium, depending on the chronicity of the event (four to five weeks) is usually described as markedly thickened with the presence of moderate degrees of hyperkeratosis and parakeratosis (3, 10). The marked proliferation of keratin poses as a physical barrier that limits the absorptive surface of the rumen mucosa leading to papillae degeneration and sloughing (19). Interestingly, contrary to findings reported in calves, none of these fawns exhibited hyperkeratosis, not even case 3 that already had some evidence of fibrosis and granulation tissue formation. This finding may be due to the differences in the time of development of rumen in calves and WTD, which ultimately impacts on the degree of manifestation of RD in these animals. Gross and histopathological analyses of other major organs in these fawns (reticulum, intestine, liver, spleen, kidney, heart, lung, skin and brain) were consistently unremarkable. The generalized depletion of fat and muscle observed in these fawns is likely a consequence of negative energy balance resulting from maldigestion, malabsorption and diarrhea (cases 2, 3, and 5).

In deer, the most common differential diagnoses of rumen ulceration without the presence of milk include viral diseases such as Bluetongue virus disease (Orbivirus) and Epizootic Hemorrhagic Disease (EHD: Orbivirus); Malignant Catarrhal Fever (Herpesvirus) (21), and Bovine Viral Diarrhea (2). Common bacterial opportunistic infections include Fusobacterium necrophorum infection and salmonellosis, amongst others (5, 6). The bacteria cultured from these fawns were concluded to be a result of secondary opportunistic invasion due to an alteration of ruminal microbiota leading to abnormal proliferation of resident bacteria. The fawns presented in this report were negative for both EHD and Bluetongue virus disease using molecular diagnostics (qPCR assay), therefore, ruling out any possibility of viral cause of diarrhea. Additionally, Clostridium perfringens isolate from case 5 was determined to be Type A, and negative for the enterotoxin gene as well. In addition, toxicologic and microscopic analyses of ruminal contents in case 4 eliminates the likelihood of cyanide poisoning and the presence of toxic plants and/or seeds.

To our knowledge, this is the first report describing clinical, gross and microscopic changes associated with reticulo-ruminal milk accumulation (RD) in WTD fawns.

Conclusion

The findings in this report provide insights into RD syndrome and associated clinical, gross, and microscopic changes in pre-ruminant WTD, and complement those of earlier studies in calves. Further experimental investigations are needed to understand the development and functionality of pre-ruminant WTD rumen, and ascertain the pathogenesis and degree of manifestation of RD syndrome in these animals.

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