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BOVINE SUMMER MASTITIS DURING VENEZUELAN RAINY SEASON: CASES REPORTS AT YARACAL, FALCÓN STATE

Mastitis de Verano en Bovinos Durante la Temporada de Lluvia Venezolana: Reporte de Casos en Yaracal, Estado Falcón

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ABSTRACT

Bovine mastitis is the disease which leads to the greatest economic losses in the dairy industry, most cases are associated with conventional pathogens, and nevertheless, a small number corresponds to infrequent microorganisms that can lead to peculiar clinical manifestations, which veterinarians may not be acquainted with so, implementation of empirical treatments is constrained. Summer mastitis belongs to this group and its etiology is correlated by the coinfection of *Peptoniphilus indolicus* and *Trueperella pyogenes*. This is the first report of bovine summer mastitis in Venezuelan herds, in the period ending August 2011 through January 2012, a surplus on rainfall was recorded in Yaracal (the northwestern area from the Country), and two consecutive outbreaks of mastitis in a bovine farm were diagnosed, where *T. pyogenes* was the causative agent isolated through bacteriological studies in aerobic conditions. This document relates the clinical manifestations and epidemiological risk factors, which were detected on the retrospective analysis in these episodes. Laboratory analyses were developed in the laboratorio de Microbiología Veterinaria de la Universidad Nacional Experimental "Francisco de Miranda", Falcón State, macroscopic and microscopic characteristics of isolated strains and its antimicrobial susceptibility profile are described, according to disk diffusion method and previously reported antibiotic assays. The recorded data were highly similar to the previous reports in the literature so, this information could be considered as highly suggestive features, which can guide veterinarians in rural areas, especially on the preliminary diagnosis and therapeutic conducts, which must be confirmed with laboratory assays.

Key words: *Trueperella pyogenes*; summer mastitis; *Peptoniphilus indolicus*; mastitis by opportunistic pathogens.

RESUMEN

La mastitis bovina es la enfermedad que conlleva a las mayores pérdidas económicas en la industria lechera, muchos casos están asociados a patógenos convencionales; sin embargo, un pequeño número se corresponde a microorganismos infrecuentes que pueden generar manifestaciones clínicas peculiares, con las cuales quizás los Médicos Veterinarios no estén familiarizados, de tal manera que la habilidad para el diseño de tratamientos empíricos podría estar comprometida. La mastitis de verano pertenece a este grupo y su etiología está correlacionada por la coinfección de *Peptoniphilus indolicus* y *Trueperella pyogenes*. Este es el primer reporte de mastitis de verano en rebaños venezolanos, durante el lapso entre finales de agosto 2011 hasta enero 2012, se registró un superávit en la temporada de lluvia en Yaracal (área noroeste del país) y se confirmaron dos brotes consecutivos de mastitis en una granja de producción bovina, en donde *T. pyogenes* fue el agente causal aislado mediante estudios bacteriológicos en condiciones aeróbicas. Este documento describe las manifestaciones clínicas y los factores de riesgo epidemiológico que fueron identificados en el análisis retrospectivo de estos episodios. Los estudios microbiológicos fueron realizados en el laboratorio de Microbiología Veterinaria de la Universidad Nacional Experimental "Francisco de Miranda"; se detallan las características macroscópicas y microscópicas de los aislamientos y su perfil de susceptibilidad a antimicrobianos, de acuerdo a los métodos de difusión en disco y reportes previos de ensayos con antibióticos. Los datos colectados fueron altamente similares con la literatura previa, por lo que esta información podría considerarse como características altamente sugestivas, la cual podría orientar a los Médicos Veterinarios en áreas rurales, y guiarles en el diagnóstico preliminar y las conductas terapéuticas, las cuales deben confirmarse con ensayos de laboratorio.

Palabras clave: *Trueperella pyogenes*; mastitis de verano; *Peptoniphilus indolicus*; mastitis por patógenos oportunistas.

INTRODUCTION

Mastitis is the disease that leads to the most important economic losses in the dairy industry [44, 48, 50]. Etiology is conditioned by determinants and predisposing factors [49, 55], corresponding to the last ones, situations which favor the infectious agents action as climatic elements, stabling conditions, pre and postmilking mammary gland hygiene, mechanized milking apparatus state, nutritional factors and immunocompetence, besides physiological variables linked to the animals like number of parity, lactation period and others [1, 3, 5, 32, 41, 44, 50].

Summer mastitis owns its name by appearance of clinical episodes in bovine (*Bos taurus* and *Bos indicus*) herds during July-October periods in North Hemisphere Countries. Nevertheless, its incidence is also increased during physiological circumstances [14, 44] and in other year seasons [28, 42]. It is an acute pathology and some authors describe it with etiology associated to *Peptoniphilus indolicus* and *Trueperella* (*Arcanobacterium*) *pyogenes* [28, 30, 51], most of the described cases in cows are related to the last one [3, 10, 14, 28-30, 36, 39], perhaps due to the technical constraints to the *P. indolicus* isolation by its condition as an strict anaerobic germ [14, 51].

T. pyogenes has a wide geographic distribution and principally inhabit mucous membranes of domestic animals [11, 13, 28, 45] except humans, whom cause an occupational disease [11, 27] and is very infrequent [45, 46]; this is an agent that can remain in fomites [31] and flies, which act as mechanical vectors [3, 28, 42, 44]. Moreover, the cited environmental conditions notably impair the sanitary settings on milking settlements [36, 50], which could lead to immunosuppressive states in animals, making them more vulnerable to opportunistic infections [5, 6, 26, 50, 52], as *T. pyogenes* [27, 46].

T. pyogenes is a bacterium with a large quantity of virulence factors [46], the most representative is a hemolytic exotoxin with cytolytic actions against neutrophils and macrophages [27, 31]. Additionally, it presents neuraminidases, fimbria and matrix-binding extracellular proteins that facilitate host tissue adhesion, it produces proteases and forms biofilms [27, 30], and these elements have been described in most of the strains isolated on clinical mastitis [57].

Phenotypic assays usually are done to the characterization of this bacterium [9, 11, 14, 45, 46, 57], whereas definitive identification tests are done on reference labs. Microscopically it is recognized as a diphteriform and pleomorphic coccobacilli, Gram positive and asporogenic [27]. The microbial growing is suspected, by inconspicuous hemolytic zones at 24 hours (h) of incubation at 37°C on bovine blood agar, thereafter at 48 h and with an exhaustive macroscopic examination, it is possible to see white pin point colonies with 1-2 mm diameter, surrounded by a more prominent total (β) hemolysis [9, 46, 57], it is partially acid-alcohol resistant and has a fermentative metabolism [35]; unlike clinical samples, cultures do not have a characteristic

odor; CAMP test is helpful to confirm the identification [13,31] and differentiation from *Arcanobacterium haemolyticum*, wich inhibit *Staphylococcus aureus* β haemolysin [47].

Antimicrobial treatment for this disease is usually unsuccessful although susceptibility *in vitro* is seen. This happens due to the massive purulent discharge and inflammatory reaction, which somehow abate medicaments effectivity [36, 57] so, most of the affected cows are early slaughtered or submitted to chemical ablation of mammary quarter [14, 28, 35, 36], even in other domestic species [12, 42], exacerbating the scenario for livestock producers, whom before taking drastic decisions try to save animals with antibiotics and alternative treatments. This phenomenon has been described in other clinical forms of *T. pyogenes* infection, including other animal species [11].

Mastitis by *T. pyogenes* is infrequent [27] and its appearance is linked to meager hygiene conditions after milking because microorganisms, like other mammary pathogens, are vulnerable to iodized solutions during nipple sealing [36, 55]. Latin America outbreaks have been described in Brazil [36, 40], in other domestic animal species [12, 37, 42] and with alternative clinical forms on bovines [9].

In this opportunity, isolation of *T. pyogenes* is demonstrated as an agent associated to an outbreak of clinical mastitis in a bovine farm at Cacique Manaure County, Falcón State (Venezuela), affecting some Carora breed animals (*B. taurus*) during rainy season ending 2011, with recurrence in other cows lots from the same property beginning in 2012. This document constitutes the first report of cases in Venezuela.

MATERIALS AND METHODS

Study area

Cacique Manaure County has approximately 225 km² area [33], even though it is one of smallest in Falcón State, it has at least 33 farms so it has a high density of bovine production units [34]. Historically, higher precipitations in the region are from September until February (mean 50 mm³ and maximum 200-275 mm³) according to the closest pluviometric station records (Coro City) [23], while mean values of lowest temperature recorded at the closest climatological station (Tocuyo de la Costa) are registered in this period as well (25,0°-25,5°C), and relative humidity slightly descends in comparison with summertime (74-78%) [24] then, coinciding with the rainy season. From August 2011 to January 2012, precipitations reached levels above historical average at the two closest pluviometric stations (in Coro and Valencia Cities), almost reaching the registered top [17-22, 25], even in Coro City was counted a pluviometric surplus of 401% with 122 mm³ over the mean [22].

Clinical descriptions and samplings

The production unit is located at Yaracal, the capital from Cacique Manaure County, which has plenty of farms dedicated to bovine livestock, being one of the most important for Falcón State [34]. The farm focused in this opportunity is committed to the breeding of several animal species, including double proposal bovines. Three hundred and twenty Carora breed cows (*B. taurus*) in lactation were submitted to double mechanical milking *per diem*, with semi-intensive management. Sanitary actions were limited to maintenance and cleaning with chlorinated solution of milking apparatus. Animals committed to dairy production composed a heterogeneous group with various age strata and different lactation times, detecting in the post-partum lot the first clinical mastitis cases focused in this report (three animals) (September 2011), while the second outbreak circa January 2012 involved two cows, also at the peripartum stage; although there were previous episodes of clinical mastitis among the bovine herds, no one corresponded to the clinical manifestations detected in this opportunity.

Affected bovines on the farm were easily recognized by macroscopic alterations in the milk (fetid odor and serous-watery aspect), without clots, pus or bloody traces, with prominent alterations on mammary glands during palpation (spontaneously draining purulent abscess and crepitaney). Only five animals were analyzed through microbiological diagnosis, because were the unique with clinical manifestations.

Aseptic samples were taken from affected cows, cleaning up mammary gland with clear water and dried with disposable napkins. Initial three spirts by manual milking were discarded, and samples of milk were taken on plastic and sterile collectors, which were maintained on refrigeration until microbiological analyses [6]. In the second mastitis outbreak, besides milk specimens, a purulent sample from mammary gland was taken in an aseptic way through aspiration with a syringe from a cow, for microbiological studies as well, disinfecting surface with iodine solution, transportation and preservation conditions were similar to the aforementioned.

Laboratory assays

Once at the laboratorio de Microbiología Veterinaria from Universidad Nacional Experimental “Francisco de Miranda”, in order to avoid more than 24 h after sampling, specimens were inoculated on primary isolation culture media on plates (MacConkey, salad mannitol, Saboraud and 5% bovine defibrinated blood agar) (HiMedia Laboratories, Mumbai, India), and incubated at 37°C in aerobic atmosphere, except blood plates which were submitted to microaerophilic conditions (CO₂ 5-10%). Cultures were daily inspected until 72 h of incubation, looking for microbial biomass or any other macroscopic evidence. Microscopic characterization was done with smears and Gram staining from culture samples, besides other phenotypic assessments (TABLE I). These procedures have been widely applied to identify this bacterium [9, 11, 13, 14, 36, 57].

TABLE I
PHENOTYPICAL CHARACTERS OF BACTERIAL ISOLATES, IN COMPARISON TO *T. Pyogenes* LITERATURE DESCRIPTIONS

Character	Documental source <i>T. pyogenes</i>	Results or positivity percentage (%)	
		Evaluated strains	
Gram positive cocobacilli, sometimes pleomorphic	8,10,12,24,50	+	+
Gelatine degradation	8,10.	-	-
Microaerophilic and CO ₂ 5-10% cultivation demand	23,26	+	+
Saboraud-dextrose agar growing	26	-	-
Aerial micelia development	26	-	-
Characteristic odor on cultures	26	-	-
CAMP	10,11	Hemolytic reaction boosted	Hemolytic reaction boosted
		Hemolytic reaction boosted	Hemolytic reaction boosted
Motility	32	-	-
Pin point colonies surrounded by hemolysis	8,10,12,50	+	+
Catalase	8,10,12,26,50	-	-
Oxidase	12	-	-
Lactose fermentation	32	94	+

To antimicrobial susceptibility tests, disk diffusion technique was applied according to Clinical and Laboratory Standards Institute (CLSI) protocols [7, 16], which have been previously used to analyze *T. pyogenes* isolates [11, 36]. There is no *T. pyogenes* specific protocol available so, *Streptococcus pneumoniae* reference methods were taken as models [8, 36, 45,

57], because both microorganisms have very similar nutritional demands, therefore, plating was done on Müller Hinton agar plus 5% bovine defibrinated blood, and incubated at 37°C with 5-10% CO₂. Antimicrobials tested were selected according to previous reports [28, 36] and availability (TABLE II).

TABLE II
ANTIMICROBIAL SUSCEPTIBILITY TESTS OF *T. Pyogenes* ISOLATES

Antimicrobial group	Antibiotic	Isolating dates and strains identification, results				
		September 2011		January 2012		Drained pus
		Milk 4678	Milk 4213	Milk 6325	Milk 3151	
Betalactam	Penicillin G 10 U (P) ^a	S	S	R	R	R
	Ampicillin 10 µg (AM) ^b	S	S			
	Piperacillin 100 µg (PIP) ^b	S	S	S	S	S
Betalactam + betalactamase inhibitors	Ampicillin/sulbactam 10/10 µg (SAM) ^b			S	S	S
	Amoxicillin/ clavulanic acid 20/10 µg (AmC) ^b	S	S			
Cephem	Ceftriaxone 30 µg (CRO) ^b	S	S	S	S	S
	Cefoxitin 30 µg (FOX) ^b	S	S	S	S	S
Carbapenems	Imipenem 10 µg (IPM) ^b	S	S	S	S	S
Phenicols	Cloranphenicol 30 µg (C30) ^b			S	S	S
Tetracyclins	Tetracyclin 30 µg (Te) ^a	I	I			
	Doxycyclin 30 µg (D) ^b	S	S	I	I	I
Quinolone	Ciprofloxacin 5 µg (CIP) ^b	S	S	S	S	S
Macrolide	Erythromycin 15 µg (E) ^b	S	S			
Lincosamide	Clindamicin 2 µg (CC) ^b	S	S	S	S	S

Legend: S, susceptible; I, intermediate; R, resistant. ^aHiMedia, HiMedia Laboratories, Mumbai, India; ^bBBL Sensi-Disc™, Becton-Dickinson, Sparks, MD.

RESULTS AND DISCUSSION

Clinical manifestations detected on animals during the two outbreaks were similar to the previously described according to literature, with swollen and hyperthermic mammary glands, with occasional suppurant abscesses [10, 14, 31, 36, 40], coinciding with previous reports in sheep (*Ovis orientalis aries*) [42] and goats (*Capra aegagrus hircus*) [12]. Besides, macroscopic appearance of mastitic milk on *T. pyogenes* infections detected in this opportunity has been formerly documented, although it is not the unique finding reported in association with this bacterium [57], including fetid milk odor [12, 28, 36]; nevertheless, this sign should not be directly associated with *T. pyogenes*, instead it could be talking in favor of a mixed infection with *P. indolicus* [28, 30, 51], which is a butyric acid producer [38] and generates a very unpleasant odor, especially when it is linked to sulphure groups. This feature was seen only on clinical samples and it disappears in subculture conditions, as recognized previously [36], maybe because in the lab procedures were given conditions to the

exclusive growing to *T. pyogenes* [14], which was principally described as a long chain fatty acids producer, that are commonly odorless [2, 56].

Macroscopic analysis of cultures at 24 h allowed to perceive tiny hemolytic zones, at 48 h whitish pin point isolated colonies were seen with a greater hemolysis, and without another colonial morphological types, discarding other species in the samples. Similar to previous papers, isolated colonies in this opportunity show traditional macroscopic characteristics [36, 57]. Microscopic visualization revealed pleomorphic Gram positive coccobacilli. No variations on phenotypic characters were registered among the five strains isolated on the two outbreaks (TABLE I).

Identification of *T. pyogenes* could be confused with *A. haemolyticum* because both have great phenotypical similarity [56], but much of the strains of the first have pyrrolidonylarylamidase, amylase and leucine aminopeptidase, besides fermentation of lactose and trehalose [35] so, these characters together with the other ones detected in this opportunity, conjugated with

clinical data, confirm the etiologic diagnosis of these outbreaks associated to *T. pyogenes*, such as other researchers have done [28, 35, 36, 57]. Other key differentiating features are the origin of microorganism and the kind of hemolysis, because *A. haemolyticum* is a commensal on human microbiota and produces a greater β haemolysis area [43, 54], meanwhile *T. pyogenes* is principally isolated on domestic animals [11, 45, 46], generates a lesser β hemolysis area [46] and CAMP test with *S. aureus* β haemolysin is different [47].

Regarding other interesting epidemiological aspects, the findings in this opportunity are coincident with ones from Brazil [10, 12, 36] and from other latitudes [14]. Although *T. pyogenes* mastitis is associated to summer time on Northern Hemisphere Countries, there are reports that relate its appearance with other physiological circumstances (drying period and short postpartum lapse) [44] and environmental conditions [14, 36], even on highly rainy and temperature seasons, maybe due to intense proliferation of flies which can act as mechanical vectors [28, 36], coinciding again with the conditions registered in this opportunity (rainfall and cows on 4-6 weeks postpartum).

Summer mastitis frequency is associated to poor sanitary conditions on milking areas, by presence of mud and high humidity corollaries to persistent rain [36], and absence of good hygienic postmilking practices, which were collectively seen on this farm, so that animals had clear signs of behavioral alterations, being refractory to circulate on mud terrain, leading to reduction of milking episodes to one per day.

Correlation between mastitis by nonconventional pathogens and rainy seasons has been previously detected in rural areas similar to Yaracal [5, 6, 52], highlighting important considerations that should be taken about immunocompetence when stressing conditions are persistently present, converting animals more susceptible to opportunistic microbes [1, 5, 6, 48], a circumstance that could be exacerbated when germ virulence factors are confronted, even they can exert immunomodulant regulations like the reported to short chain fatty acids produced by *P. indolicus*, although this bacteria was not registered in these episodes at Yaracal, its etiologic association could be inferred considering the clinical evidence [4, 53].

This document is a retrospective analysis of recorded clinical cases, with detailed report of several findings linked to two consecutive clinical mastitis outbreaks, which have been diagnosed as summer mastitis by *T. pyogenes*. According the analysis of overall evidences, the conjunction of the reviewed elements could be highlighted as a potential "marker" of the disease; nevertheless, systematic studies on epidemiological risks factors specific to mastitis by *T. pyogenes*, must be undertaken to corroborate these ideas, especially about the possibility *P. indolicus* coinfection, which could dramatically change the considerations to design empirical antibiotic treatments. It is necessary to mention the report of Ribeiro *et al.* [45], who did not find statistical association between general *T. pyogenes* infections and age, gender and seasonality.

Regarding antibiotic susceptibility test, few differences on strain profiles were perceived among the two episodes, including the diameter on disk diffusion tests (TABLE II). The evaluation of antimicrobial susceptibility on *T. pyogenes* strains isolated on bovine mastitis is not frequent [57], in this opportunity the assays were done to guide veterinary physician about therapeutic possibilities, getting a general sensibility to different antimicrobials groups.

β lactamics (AM, P y AmC) have proven *in vitro* action against *T. pyogenes* [11, 28, 36, 44, 57] and are traditionally applied on clinical veterinary activities, with few reports of resistant isolates [11, 36, 46], as could be seen on second outbreak (TABLE II), the exceptions are Penicillin G and Oxacillin [11, 44, 46]; nevertheless, Ribeiro *et al.* reported different susceptibility. Neither cephem resistance has been observed [28, 36, 44] nor to fenicolos (Florfenicol and C30) [28, 36], as could be seen in this report. About the rest of antimicrobials evaluated (TABLE II), partial concordances were detected to previous documents, i.e. sensible and intermedium strains were registered to tetracyclines like findings on Brazil [36], but in other latitudes a high frequency of tetracyclines resistance has been recorded [28], even linked to macrolides resistance (E) [57]; low quinolones effectivity were seen too (enrofloxacin) [36] and intermedium susceptibility to CC [28], being different to TABLE II data.

Experts recommend implementing parenteral (intravenous) treatment on acute cases like summer mastitis [15, 46], because intramammary or local administration might not be the adequate route, due to intense pus discharge and inflammatory reaction: Considering that *in vitro* assays can show a wide spectrum of susceptibility to different antimicrobial families [28, 44], will be appropriate to evaluate antibiotics with high bioavailability on mammary gland or milk as P, sulfonamides and macrolides like E or tylosin [15]; nevertheless, there are several reports of *T. pyogenes* strains resistant to the them [11, 44, 46] so, the use of these antibiotics should be considered according to results of respective antimicrobial susceptibility tests.

On the other hand, there are specialists who prefer chemical ablation of mammary quarters as a more traditional alternative in cases where pharmacological treatment fail [36]. Nevertheless, in most of the cases premature culling is the irremediable procedure [14, 36], as occurred on the episodes at Yaracal, where animals were treated with diverse antibiotics (oxitetracyclin, cephem and penicillin) and there were not satisfactory results, in a similar way, García *et al.* [11] reported a failed antibioterapic treatment in a pig with multiple abscesses.

CONCLUSIONS

This is the first diagnostic report of summer mastitis on Venezuelan bovine herds, being highly similar to previous ones about clinical manifestations, epidemiological risk factors and phenotypical characteristics for one of the etiologic agents (*T.*

pyogenes). Although bacteriological protocols did not consider methodologies to detect anaerobic bacteria, clinical signs let to suspect the association with *P. indolicus*, given a highly suggestive scenario which could preliminary helps veterinarians in rural areas to have a presumptive diagnosis, who must corroborate their suspects with laboratory tests.

Antimicrobial susceptibility profiles of isolates show sensibility to numerous antibiotics, although they were not effective *in vivo*, in a similar way with previous clinical reports, maybe due to the absence of a complementary treatment to combat anaerobic germs.

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