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Assessment of cow and farm level risk factors associated with *Ureaplasma diversum* in pasture-based dairy systems - A field study

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ABSTRACT

Potential risk factors for *Ureaplasma diversum* in the vaginal mucus of 1,238 dairy cows were included in a multivariate logistic regression model, based on the cow level (i.e., granular vulvovaginitis [+GVV], yearly milk production [4500 kg or more], pregnancy, predominance of *Bos taurus* [+*Bos Taurus*], score of corporal condition [at least 2.5], concomitant positivity for *Escherichia coli* [+*E.coli*]), and farm level i.e., milking room hygiene (-Milking room), dunghill location, and replacement female). *Ureaplasma diversum* was present in 41.1% of the samples. Independent risk factors for *U. diversum* were +GVV (odds ratio [OR], 1.31); +*Mycoplasma spp* (OR, 5.67); yearly milk production (4500 kg or more) (OR, 1.99); +*Bos taurus* (OR, 1.68); +*E. coli* (OR, 4.96); -milking room (OR, 2.31); and replacement females (OR, 1.89). *Ureaplasma diversum* vaginal colonization was strongly associated with *Mycoplasma spp.*, *E. coli*, and number of pregnant cows.

Key words: dairy cow, exposure factors, genital ureaplasmosis, mollicutes.

INTRODUCTION

Ureaplasma diversum is an opportunistic mollicute associated with granular vulvovaginitis (GVV) in cattle (Buzinhani et al. 2007). GVV is also associated with the presence of *Mycoplasma bovis* and *Mycoplasma bovigenitalium* (Buzinhani et al. 2007, Lysnyansky et al. 2009). *Mollicutes* may be found alone or in association with other microorganisms such as *Pasteurella multocida* and *P. haemolytica*, which have been associated with respiratory diseases in calves (Hirose et al. 2003), and may play a role in the severity of pathological processes and in predisposing an animal to infection of the lower respiratory tract (Virtala et al. 1996). In other species, this synergistic role was studied in the airsacculitis in gnotobiotic chickens (Springer et al. 1974) and in women with spontaneous abortion or preterm delivery (Raddi and Lorencetti 1998).

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In the cow one of the signs of the infection by *Mollicutes*, the GVV, is an inflammation characterized by hyperaemia and nodules containing lymphocytes and plasmocytes in the lamina propria of the mucosa. Cows may present with GVV three to six days postbreeding (Doig et al. 1979). GVV can be classified into different grades, according to the amount and distribution of nodules in the mucosa and the grade of hyperaemia (Gambarini et al. 2009).

It is considered a commensal organism, although it has been associated with GVV outbreaks (Lysnyansky et al. 2009). Experimental infections and clinical isolates from commercial herds indicate that mollicutes are associated with GVV and significantly affect reproductive efficiency by increasing the number of days of open breeding and the number of repeat breeding cows (Nascimento et al. 2005, Buzinhani et al. 2007, Gambarini et al. 2009). Besides the reproductive tract, *U. diversum* inhabits all mucosal membranes and causes a subclinical respiratory infection and bronchopneumonia in calves (Brown 2010). In Cuba, it was detected in pneumonic lung samples of pigs (Burgher et al. 2014). In Brazil, a previous study adopted a multivariate logistic regression and observed that the presence of vulvar lesions and the use of progesterone devices during reproductive protocols were significantly associated with the presence of *U. diversum* (Gaeti et al. 2014). Granular vulvovaginitis in cows has been associated also with bovine herpesvirus type 1 (BHV-1) (Wentink et al. 2000, Van Schaik et al. 2002, Anderson 2007), but microbiological and serological assessments of cows presenting different grades of GVV show that it is not an exclusive reaction to BHV-1 (Blum et al. 2007).

U. diversum can be detected inside cells or adhered to their surfaces (Marques et al. 2010) and induced significant TNF-alpha production in the uterus of mice (Silva et al. 2016), indicating that the colonization of the reproductive tract of females may alter the homeostasis of the uterus microenvironment (Marques et al. 2016). Recent studies using the metagenomic to evaluate the progression of the uterine microbiota from calving until establishment of metritis have shown that among 824 bacteria genera found in uterine samples of metritic and non metritic cows, *Ureaplasma* was present in 5.2%, with a relative increasing in the number of positive samples for this bacterium from 0 to 2 days post partum, and then decreasing from 2 to 6 ± 2 days post partum (Jeon et al. 2015).

Cell invasion by the mollicutes has been described in phagocytic and nonphagocytic cells, and the recurrence and/or chronicity of genital mycoplasmosis can be explained by immune escape mechanisms (Rottem 2003). This may enhance the understanding of biological changes in the mollicutes as a mechanism of adaptation to the host and understanding its transmission, which was previously reported in studies investigating the invasion of *Trichomonas vaginalis* by *Mycoplasma hominis* (Vancini and Benchimol 2008). The findings that the reference strains and clinical isolates of *U. diversum* are able to invade human epithelial type 2 (HEP-2) cells one minute after infection is the fact that allows identifying the agent as a cellular invader in mammals and explains its impact on bovine mycoplasmosis (Marques et al. 2010).

Little is known about the possible risk factors associated with genital ureaplasmosis in bovines, which may occur by venereal transmission during natural mating or artificial insemination (*U. diversum* has been detected in bovine semen straws), indicating a large-scale risk of dissemination in herds (Marques et al. 2009). Other potential routes of dissemination are embryo transfer (Britton et al. 1988), contact between confined animals (Sanderson et al. 2000) or contact between animals maintained in groups for reproductive management (Gambarini et al. 2009), and during milking (Maunsell and Donovan 2009). Risk factors can

be defined as the measurable characterization of diverse conditions that contribute to or increase the probability of a disease and can be related to the individual, the environment, or social media. For lactating cows, high production, parity, and calving season are risk factors for reproductive disorders, increasing the number of days open, services per conception, and the rate of involuntary culling (Gröhn and Rajala-Schultz 2000). Identifying risk factors should take into consideration the differences in the occurrence of the problem, based on comparisons between individuals or groups with different levels of a proposed factor (Sargeant et al. 1998).

Studies involving *Bos taurus* females in a tropical environment show that breed, parity, and age increase the chances of dairy cattle testing positive for *U. diversum* (Leon et al. 1995). The identification of potential risk factors may assist in the decision-making process to minimize the effect on the animal and to manage its dissemination in the herd (Givens and Marley 2008).

This study was performed to verify the presence of *U. diversum* in the vaginal content of cows with and without the typical lesions of granular vulvovaginitis, and to evaluate its correlation with potential risk factors inherent to the animal and to the production system.

MATERIALS AND METHODS

This study was conducted according the Brazilian guideline for the care and use of animals in education and scientific research activities (DBCA) and was approved by the Ethics Committee for the use of animals of the Federal University of Goias, Brazil.

STUDY SITE

Fifteen commercial pasture-based dairy farms (DFs) were visited, and 1,238 cows were sampled. The DFs were located in the middle west region of Brazil, the Cerrado biome, located in two mesoregions (central and south): latitude between -16° 01’ 13’’ and 17º 47’ 53’’; longitude between 49° 18’ 40’’ and 50º 55’ 41’’; altitude between 716 and 948 meters above sea level; average temperature between 23°C and 31°C; and rainfall between 50–180 mm.

SAMPLING AND ASSESSMENT OF RISK FACTORS

For this study, the DFs were selected, according to the following criteria: pasture-based system, participation in monitoring programs by a veterinarian, use of artificial insemination, strict observation of the compulsory prophylactic calendar, and at a distance of maximum 200 km from the University campus. All DFs were engaged in an extension program conducted by the same milk industry. In the DFs, all lactating cows with 90–100 days in milk and parity between 2 and 3 were sampled, except cows with a history of abortion, premature calving, stillborn birth, placenta retention, and/or puerperal metritis in the previous pregnancy.

The DFs were visited by the same team that collected and processed the vaginal mucus. The DFs were evaluated under two aspects, based on potential risk factors as follows:

- Cow level, in relation to the presence of GVV, yearly milk production (at least 4500 kg), pregnancy (+, –), predominance of *Bos taurus* in crossbreeding (+*Bos taurus*), score of corporal condition (SCC) of 2.5 or greater, and positive cultures of the vaginal content for *Escherichia coli* (+*E. coli*).
- Farm level, relating to hygiene in the milking room [-Milking room, if one of the four points failed – removal of feces during milking, use of paper towel to clean teats, washing the milking parlor only after all groups of cows were milked, use of detergents to clean the milking room),

dunghill location (<50 m), and purchase of replacement females (in the last six months).

After the sample processing, one additional factor was added to the inherent conditions of the cows: concomitant positive isolation of *Mycoplasma* spp (+*Mycoplasma*).

In all DFs, the same veterinarian performed sampling during milking. Each cow was evaluated and sampled once. The vulvovaginal mucosa was assessed for the presence of GVV and classified as "absent" or "present", as adapted from Gambarini et al. (2009). The subsequent procedure consisted of dry cleaning the perineal and vulvar regions and collecting vaginal mucus by using two sterile swabs rubbed on the mucosa. The first sample was placed in an A_{3xB} transport medium (Cunha et al. 1987) and used for the isolation of Mollicutes, and the second sample was placed in thioglycolate medium for the isolation of *E. coli*. All samples were processed within 24 hours of collection. For the isolation of *U. diversum* and *Mycoplasma* spp., aliquots were inoculated in Ub medium, agar Ub, and agar Hayflick, incubated, and evaluated as described by Ruhnke and Rosendal (1994). Briefly, agar plates were incubated for up to 15 days, at 37°C in a microaerophilie environment (atmosphere of 85% $N_{2,}$ 10% CO_2 and 5% $O_{2)}$. The broths were incubated in aerobic atmosphere during 5 days at 37°C.

The tubes and plates were examined daily to assess the urease activity and presence of small dark brown-colored granular colonies (*U. diversum*) and to identify "fried egg" colonies (a characteristic of the mycoplasmas), which were confirmed by Dienes staining. For the isolation of *E. coli,* the samples were processed according to Oliveira (1995).

STATISTICAL ANALYSIS

The descriptive statistics was generated, and the distribution of the frequency of animals positive or negative for *U. diversum* and potential risk factors were assessed. Possible collinearity between variables was determined by the chi-squared test. Multivariate analysis was performed by using a logistic regression model that included +GVV; SCC of 2.5 or greater; +*Mycoplasma* spp.; yearly milk production of at least 4500 kg; +pregnancy; +*Bos taurus*; +*E. coli*; -Milking room; dunghill location less than 50 m; and the purchase of replacement females. Data analysis was performed by using the software R (R Core Team, 2013) with the *glmer* function of the lme4 package (Bates et al. 2013).

RESULTS

Out of the 1,238 vaginal mucus cultures, 41.1% tested positive for *U. diversum*. The prevalence of *U. diversum* for the DFs ranged between 20% and 79%. Table I shows the frequency of the distribution of the categorical variables that were assessed as potential risk factors for *U. diversum* positivity. Table II shows the odds ratio (OR) and the P values resulting from the model of multivariate logistic regression for the 1,238 cows assessed for the presence of *U. diversum*. +Pregnancy and a SCC of 2.5 or greater did not show significant results in the first model ($P > 0.05$), although the frequency of samples positive for *U. diversum* has been higher than 40%.

Independent risk factors for *U. diversum* associated with the cow level were +GVV (OR, 1.31, $P < 0.001$), +*Mycoplasma spp* (OR, 5.67, $P < 0.0001$), yearly milk production of at least 4500 kg (OR, 1.99, $P < 0.05$); +*Bos taurus* (OR, 1.68, $P < 0.001$), and +*E. coli* in the vaginal mucus (OR, 4.96, $P < 0.001$). This means that there are significant associations between these variables and positive vaginal samples for *U. diversum*. The most significant results were that the presence of *Mycoplasma* spp was 5.67-fold higher in females positive for *U. diversum* and 4.96-fold higher in cows with *E. coli* positive vaginal mucus samples.

TABLE I
Distribution of risk factors related to vaginal positive samples to *Ureaplasma diversum* on the 1,238 evaluated cows and 15 Dairy Farms Systems.

			Ureaplasma diversum			
			Positive		Negative	
	Level	N	n	(%)	n	(%)
Number of sampled cows		1,238	509 (41.1)		729 (58.9)	
Cow level						
+GVV	No	481	200 (41.6)		281 (58.4)	
	Yes	757	309 (40.8)		448 (59.2)	
+ *Mycoplasma* spp	No	925	276 (29.8)		649 (70.2)	
	Yes	313	233 (74.4)		80 (25.6)	
Milk production ≥4500Kg/milk/year	No	258	127 (49.2)		131 (50.8)	
	Yes	980	382 (39.0)		598 (61.0)	
+Pregnancy	No	823	338 (41.1)		485 (58.9)	
	Yes	415	171 (41.2)		244 (58.8)	
+ Bos taurus	No	531	167 (31.5)		364 (68.5)	
	Yes	707	342 (48.4)		364 (51.6)	
SCC>2.5	No	833	333(39.9)		500 (60.1)	
	Yes	405	155(38.3)		250 (61.7)	
+*E. coli*	No	806	255(31.6)		551 (68.4)	
	Yes	432	304(70.3)		127 (29.4)	
Farm level						
-Milking room	No	842	316 (37.5)		526 (62.5)	
	Yes	396	193 (48.7)		203 (51.3)	
Dunghill <50m	No	840	351 (41.8)		489 (58.2)	
	Yes	398	158 (39.7)		240 (60.3)	
Purchase of replacement females	No	847	350 (41.3)		497 (58.7)	
	Yes	391	159 (40.7)		232 (59.3)	

TABLE II
Results of multivariable logistic regression analysis of risk factors between vaginal positive samples to *Ureaplasma diversum* and independent variables on the 1,238 evaluated cows and 15 Dairy Farms Systems.

Variable	OR	C.I.		P-value
Cow level				
+GVV	1.31	1.12	1.54	< 0.001
+ *Mycoplasma* spp	5.67	4.16	7.72	<0.0001
Milk production ≥4500Kg/milk/year	1.99	1.09	2.08	<0.05
+Pregnancy	0.95	0.72	1.24	NS
+*Bos Taurus*	1.68	1.23	2.05	<0.001
SCC≥2.5	0.89	0.69	1.15	NS
+*E. coli*	4.96	3.21	7.66	<0.001
+*E. coli* and Pregnancy*	5.15	3.46	7.68	<0.001
Farm level				
-Milking room	2.31	1.71	3.12	<0.001
Dunghill <50m	1.71	0.52	1.95	NS
Purchase of replacement females	1.89	1.38	2.57	<0.001

* Mantel-Haenszel chi-squared test.

In the final multivariate logistic regression model, the probability of samples positive for *U. diversum* was 73.1% for cows with +GVV, +*Mycoplasma* spp., pregnancy+, yearly milk production of at least 4500 kg, +*Bos Taurus,* and +*E. coli.* The results showed a positive association between *U. diversum* and +*E. coli* (OR 4.96, 3.21-7.66, P < 0.001), and between +*E. coli* and +pregnancy (OR 1.78, 95% CI, 1.32-2.10, P < 0.01). Therefore, to address confounding factors, the Mantel-Haenszel chi-squared test was applied to stratify by the variable of +pregnancy with regard to the presence of *U. diversum* and +*E. coli*. The adjusted OR was 5.15 (95% CI, 3.46–7.68, P < 0.001), which showed a significant association between these three variables, that mean, the probability of *U. diversum* positive vaginal mucus is 5.15-fold higher in pregnant cows presenting also *E. coli* in the vaginal mucus. At the farm level, independent risk factors were the -Milking room (OR 2.31, P < 0.001) and the purchase of replacement females (1.89, P < 0.001). The probability of cows positive for *U. diversum* was 43% for -Milking room and 41% in the farms that purchased replacement females. No association was found with a dunghill located less than 50 m.

DISCUSSION

Data are often contradictory concerning the clinical importance of the involvement of ureaplasmas in reproductive disturbances in humans and animals. The lack of a cell coat, a saprophytic and facultative anaerobic condition, its high affinity for an epithelial surface, and its resistance to temperature changes favor its survival and dissemination. However, in many studies, differences in serotypes and in the pathogenicity of the strains make interpretation difficult. The average percentage (41.1%) of cows positive for *U. diversum* in the present study is similar to the percentage previously reported in Brazil and in other countries. In cattle, the presence of *U. diversum* has been reported in many countries (Canada, Costa Rica, France, England, United States of America, Brazil), and most recently in Australia, where researchers hypothesize that the lack of association between reproductive diseases and *U. diversum* detection may be caused by missing data or by misdiagnosed problems (Argue et al. 2013). Some research teams have linked the identification of this bacterium with abortion, after performing additional tests in animals that had not been diagnosed through routine procedures (Watson et al. 2012). This confirms data published in Finland (Syrjälä et al. 2007).

In the present study, all DFs had positive cows for *U. diversum* and GVV, and of the 509 samples positive for *U. diversum*, 200 (39.8%) samples were obtained from cows without GVV; of 729 samples negative for *U. diversum*, 448 (61.6%) had GVV.

In Canada, the evaluation of dairy cows with and without GVV showed a frequency of 43.3% for *U. diversum* (Mulira et al. 1992); in France, 40% of the samples were positive for *U. diversum*, although without a relationship with GVV (Le Grand et al. 1995). In the tropical climate conditions of Brazil, positive isolation of *U. diversum* was reported for the first time in 2000 (Cardoso et al. 2000) in mucus from cows with reproductive disorders and GVV with a frequency of 52.6% in Holstein cows and 23.7% in Jersey cows. In Austria, *U. diversum* was present in all studied farms with 35.5% of the animals testing positive for the organism, which was associated with vaginitis (Petit et al. 2008). Previous studies reported a predominance of mild GVV in more than 50% of repeat breeding dairy cows with samples positive for *U. diversum* associated with this lesion grade (Oliveira Filho et al. 2005). In beef heifers, there was a predominance of mild to severe GVV that was also associated with *U. diversum* (Gambarini et al. 2009). There was an association between mild GVV and *U. diversum*-positive samples in replacement beef heifers, but a tendency was verified for higher calving rates in

heifers negative for *U. diversum* (Sanderson et al. 2000).

The local reaction of the macroscopic aspect of the GVV may be related to the virulence of the strain. The immunological response depends on the virulence of the strain and the local mechanisms of cell response. These results in individual variations related to breed, productivity, and other concomitant infections. The preferred attachment site of *U. diversum* is epithelial tissue, particularly respiratory and genital epithelial cells. *U. diversum* can therefore be considered a surface parasite (Rottem 2003). In the present study, the presence of mollicutes in vaginal mucus was assessed by cultured samples. However, using polymerase chain reaction (PCR) increased the detection of *U. diversum* from 25% to 37.5%, which is the percentage of positive samples from cows with reproductive disorders (Buzinhani et al. 2007). This indicates that these bacteria may be present in a larger number of cows in the studied DFs. An ascending infection can be one mechanism of choice for uterine and fetal contamination; this combination would be harmful for reproductive health and must be evaluated in reproductive failures, abortion, stillborn, weak newborn calves, and bronchopneumonia in young calves (Brown 2010). Mild GVV and the presence of *U. diversum* indicates the acute phase of vulvovaginitis, as previously reported in experimental infections by uterine and cervical inoculation in heifers (Doig et al. 1980). A +GVV was most frequent in pregnant and +*Mycoplasma* spp cows.

The present data were obtained from dairy cows during a very particular phase of their reproductive cycle because all were 90–100 days in milk, had regular estrus cycle intervals after calving, and were under a regular program of artificial insemination. The inclusion of the variable *E. coli* in the analysis also increased the odds of positivity to *U. diversum* in cows with +*Mycoplasma* spp., showing that the colonization of the vaginal mucosa with *E. coli* may favor the proliferation of *U. diversum* and mycoplasmas. When identifying *E. coli* in vaginal samples positive for *U. diversum*, a significant OR indicates an interaction between *E.coli* and the mollicutes, which has been previously reported for mollicutes and streptococci isolated from ulcerative lesions of the genital tract of equines (Spergser et al. 2002) and *Pasteurella multocida* in lesions of respiratory disorders caused by mycoplasmas in cows (Maunsell and Donovan 2009). The synergism between Gram negative bacteria and the ureaplasmas may exist because of the protein similarity of their membranes (Brenner et al. 1997). Cows with metritis showed a predominance of *E. coli* strains in the vaginal bacterial microbiota with a marked increase of *E. coli* in vaginal samples from infected postpartum cows (Wang et al. 2013). Under natural conditions the vaginal microbiota of cows is stable and may protect the animal from potential pathogenic saprophytic microorganisms. A very low level of Enterobacteriaceae was detected in the heifers and increased during the growth of the animal, which would be associated with the establishment of hormonal cycles resulting from puberty (Otero et al. 2000).

The inclusion of the variable +Pregnancy in the stratified analysis increased the OR (5.15), suggesting that the variable combination of +pregnancy and *E. coli* play an important role on the probability to obtain *U. diversum*-positive samples in the studied cows population. Immunological suppression to minimize rejection of paternal antigens and escape mechanisms against phagocytosis present in mollicutes may explain this finding (Kwiecien and Little 1991). Pregnant females are immunologically suppressed with reduced circulation of T cells and B cells in the reproductive system to reduce the risk of rejection to paternal antigens acquired during conception. The mollicutes have escape mechanisms against phagocytosis while they are in an extracellular medium (e.g., in the mucosa of

the reproductive tract), reducing the possibility of bacterial recognition when they later invade the cells (Kwiecien and Little 1991).

Bos taurus cows, especially the Jersey breed, have a greater probability of being positive for mollicutes. All farms visited in the present study were pasture-based systems in which *Bos taurus* × *Bos indicus* crossbreeds predominate. Thus, racial characteristic was classified with regard to the predominant breed (i.e., taurine or zebuine). The results demonstrate that the cows that are predominantly *Bos taurus* are 1.68 times more likely to be positive for *U. diversum* ($P < 0.001$) than those predominately *Bos indicus*. This risk factor was identified in *U. diversum* infections (León et al. 1995, Cardoso et al. 2000, Bey 2006). When the results of *U. diversum* are compared among *Bos taurus* and *Bos indicus,* the frequency was higher between Nellore females, followed by Jersey cows and Holstein cows (Cardoso et al. 2000). The milk production was not high in any of the visited farms, although cows of predominant *Bos taurus* breed tend to produce more milk and are more likely to demonstrate stressed behavior (Gröhn and Rajala-Schultz 2000, Gábor et al. 2008), which contributes to the manifestation of diseases or functional alterations.

In this study, three factors on the farm level were assessed as probable variables related to the higher frequency of samples positive for *U. diversum.* These factors were selected because of the possibility of using the same evaluation profile for all farms, unlike other factors that are related to sanitary conditions (e.g., application of pre- and post-dipping or protocols of treatment), which differ according to the procedures adopted and the products used. Two variables were confirmed as risk factors inherent to the system. Poor hygiene in the milking room showed the highest odds with a 2.31 times increased risk of the cows being positive. Mollicutes are very susceptible to adverse environmental conditions and respond by cell lysis to osmotic shock caused by detergents (Chambaud et al. 1999). However, cleaning the milking room with only water or with an inadequate amount of detergent is insufficient to eliminate the agent and is a risk factor in the maintenance of microorganisms in the ambient environment (Vairamuthu et al. 2010). It also increases the risk of their dissemination by the hands of the milker (Al-Momani et al. 2008). The mollicutes can adapt themselves to different hosts, and mutations have been reported as a survival mechanism (Yogev et al. 2002).

M. bovis has been isolated from bovine feces up to 37 days after contamination (Caswell and Archambault 2007). The transmission to other hosts from contaminated dung has been suggested (Madoff et al. 1979, Pitcher and Nicholas 2005). The urea present in the urine incorporated in the material sent to the dunghills may contribute to the survival of *U. diversum*, which uses it as a nutritional source. Ureaplasmas are microaerophilic, and although the optimum temperature for their growth is 37°C, they can grow in a temperature range of 22–42°C (Brown 2010). In regard to the environmental temperature for most of the year in tropical regions, the adaptation of this microorganism may favor its survival in the environment of the milking room and in the dunghill.

The odds ratio for cows being positive to *U. diversum* was 1.89 times higher in DFs that purchase replacement cows ($P < 0.001$). The purchase of an animal increases the risk of spreading diseases because new animals may already be contaminated, because of the stress caused by transportation and because adaptation temporally inhibits the immune system (Van Schaik et al. 2002). Clinical signs of genital infection by mollicutes are usually reported when the female is been evaluated to be introduced or to return to the reproductive management, that is, when she is considered apt to reproduction. The infected cow is included in the routine reproductive management and repeatedly returns in estrus, after

artificial insemination or natural mating. Even GVV (which researchers indicate as a signal of genital infection by mollicutes), if not severe enough to cause discomfort to the animal, would not be considered a primary cause of a return to estrus. Thus, the replacement female may act as a source of dissemination.

In conclusion, the colonization of the vaginal mucosa with *U. diversum* was strongly associated with the presence of *Mycoplasma spp.* and *E. coli*, and with positive pregnancy in the studied population. The results showed that screening for risk factors at the cow and farm level should allow better assessment of the risk of obtaining cultures positive for *U. diversum* in dairy cows. Cows presenting GVV probably are infected with *Mollicutes*, the predominance of *Bos taurus* in the crossbreed favors the vaginal colonization by these bacteria and, a new finding, that there is a bacterial synergism action between *Mollicutes* and *E. coli* would allow future studies to establish prophylactic and control measures, and a better understanding of asymptomatic reproductive infections in dairy cows and their newborn calves. On the farm level, the association of *U. diversum* with two hygiene-related variables highlights the importance of the periodical screening of the dairy system.

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