

Interference of *Mycoplasma* spp. or *Ureaplasma* spp. in Ovine Semen Quality

Lilian Gregory^{1,*}, Huber Rizzo¹, Natália C. Gaeta¹, Gabriela Tortorelli¹, Maristela V. Cardoso²,
Elena Mettifofo³, Melissa Buzinhani³, Jorge Timenetsky³

¹Department of Medical Clinic, Faculty of Veterinary Medicine, University of São Paulo, 05508-270, Postcode, Brazil

²Laboratory of Bacterial Reproduction, Center for Animal Health, Biological Institute of São Paulo, São Paulo, 04010-970, Brazil

³Mycoplasma Laboratory, Institute of Biological Sciences, University of São Paulo, São Paulo, 05508-900, Brazil

Abstract The presence of mycoplasma in ovine semen was associated in 9.09% (3/33) to the microscopic and macroscopic alterations of this fluid. *Mycoplasma* spp. was isolated in 36.36% (12/33) from semen samples while *Ureaplasma* spp. was isolated in 12.12% (4/33). The mollicute infection rates in studied semen samples indicated that the diagnosis of these bacteria in ovine must be a routine procedure for the quality of this biological product.

Keywords Mycoplasma, Ureaplasma, Ovine Semen

1. Introduction

The reproductive disorders in animals interfere the economic investments in cattle breeding. The related diseases may be caused by handling deficiencies, environmental and genetic influences and infectious agents. The urogenital infections may also be caused by some Mollicutes and *Mycoplasma* spp. and *Ureaplasma diversum* are mostly detected or isolated and associated with the reproductive disorders in bovines [1,2].

In 1955 Albertsen, in Denmark raised the possibility that the antibiotic-resistant microorganisms were responsible for the persistent low fertility bulls [25]. The first mycoplasma was isolated in 1898 by Nocard and Roux in France in a clinical material obtained from a bovine during an outbreak of contagious bovine pleuropneumonia.

A bovine *Ureaplasma* was isolated firstly in 1978 and by the time many bovine reproductive disorders were controversially associated because this mollicute was also isolated from healthy animals. This bacteria was studied through experimental and natural infections in bovines causing abortion, infertility and vulvovaginitis syndrome [3,4,5,6]. Ball and Mc Caughey [7] mentioned that the presence of mycoplasmas in vulvar or cervix side may be normal but their isolation from cervix or uterus was considered a pathological condition.

The association of *Mollicutes* with reproductive disorders in caprines and ovines was mentioned less frequently [8-10]. *Mycoplasma* serotype 11-strain 2D, is a not yet classified

strain and was related with cases of vulvovaginitis and reproductive disorders in Australia, USA, India, England, France and Nigeria [10]. Other species were isolated from reproductive tract of ovine and caprine with circumstantial evidences of pathogenesis. In this context are included *M. mycoides* subsp. *mycoides*, *M. bovis genitalium*, *M. agalactiae*, *M. mycoides* subsp. *capri*, *M. arginini*, *M. alkalescens* e *Acholeplasma* spp. [10,11,12]. Fe *et al.*, [13] obtained three positive PCRs and cultures for *Mycoplasma* spp. in 146 samples of caprine semen. Using serological and PCR methodology the isolates were identified *M. agalactiae*.

Livingston *et al.*, [14] and Ball *et al.*, [7] inoculated in caprines and ovines field isolates of ureaplasma recovered from other diseased animals and related them to the serotype IX as causative of infertility and abortion in sheep.

Actually seven species of ureaplasma are recognized. They are human, bovine, canine, feline and avian origin. It was described other five species, but they also did not received yet a nomenclature [15]. These isolates were included in those recovered from ovine or caprine and nine described serotypes. Their DNA and some polypeptides pointed a similarity with *U. diversum* [8,16,17]. The pathogenicity of these microorganisms in reproductive disorders in caprines and ovine are unknown.

The adherence of mentioned Mollicutes to the bovine spermatozoa is the initial step in this host-parasite relationship. Nicholas *et al.* (1999) described decreased motility of spermatozoa in semen of bulls belonging to herds infected with *Mycoplasma* ovine/caprino serogroup 1. These animals presented low fertility rates, and degeneration of the tail in cases of orchitis. AK *et al.* (1995) inoculated experimentally *M. agalactiae* and observed a decrease of volume, activity, motility and semen

* Corresponding author:

lgregory@usp.br (Lilian Gregory)

Published online at <http://journal.sapub.org/microbiology>

Copyright © 2012 Scientific & Academic Publishing. All Rights Reserved

concentration, with an increase in abnormal sperm.

Some species of *Mycoplasma* and *Ureaplasma diversum* were associated with seminal vasculitis, balanoposthitis, epididymitis,[27] other functional disorders and morphology of spermatozoa[28,29] including a reduced motility.[30,31,[32,33,34,35,36]

The virulence mechanisms by which organisms Family Mycoplasmataceae cause problems for cells are related to interaction with the immunological system, changes in humoral and cellular immune response and the production and induction of cytotoxic components such as ammonia and peroxidase.[38]

Mycoplasma spp and *Ureaplasma diversum* has a worldwide distribution and can be spread through international trade of animals, semen and industrial products for transferring embryos.[30,37,38]

In this view it was compared the presence of mycoplasma in ovine semen and the influence of this infection in macroscopic and microscopic aspects of this fluid.

2. Material and methods

2.1. Microorganisms

Ureaplasma diversum ATCC49783, *U. urealyticum* T-960, *Mycoplasma capricolum*, *M. conjunctivae* HRC581, *M. arginini* G230, *Acholeplasma laidlawii* PG-8.

2.2. Sampling

Ovine semen was obtained by electro-ejaculation from 33 ovine (breed Santa Inês, Poll Dorset e Texel). The animals were from three properties of a region named Piedade/SP-Brasil. The clinical samples were aliquoted in vials for culturing, microscopic and macroscopic analysis.

2.3. Mycoplasma culturing and characterization

The ATCC strains of mycoplasma and ureaplasma were inoculated respectively in SP4 and UB agar. The UB was with manganese sulphate. The UB broth was adapted from U9C because was added with de CMRL-1066 as in SP4. Semen samples were diluted previously in SP4 or UB broths to 10^{-3} and inoculated in respective media. The incubation was at 37°C. The inoculated UB broth were in aerobiosis and the SP4 broth were at 10% of CO₂. The cultures were incubated for 21 days and observed for the production of small fried egg colonies for *Mycoplasma* spp or dark brown granulated colonies for *Ureaplasma* spp. The ureaplasmas were also characterized for alcalinization of UB broth due urea hydrolysis by urease activity[5,18].

2.4. Semen Analysis

The semen samples were observed for physical, morphological and cellular aspects. The used parameters were the mentioned by Colégio Brasileiro de Reprodução Animal[19].

2.5. Statistical analysis

Risk estimative, Odds Ratio, was obtained by software GraphPad InStat, version 3. for Windows.

3. Results

The reference strains grew in SP4 and UB media. *Mycoplasma* spp. was isolated in 12 (36.36%) semen samples while *Ureaplasma* spp. was isolated in four (12,12%) samples. It was observed an association of *Mycoplasma* spp e *Ureaplasma* spp. in 9.09% (3/33) with semen alterations in some cases. All positive cultures to mycoplasma were not associated with semen alterations. The *Mycoplasma* spp. isolates produced “fried egg” colonies and was not retained by 0.45µm porosity membrane. The *Ureaplasma* spp. isolates also produced typical colonies and increased the pH of UB broths. Four semen samples were contaminated and did not allow the mycoplasma isolation. Tables 1, 2 and 3, presents the results from cultures and risk rates for the alteration of semen aspects.

Table 1. Estimated risk regarding to association between *Mycoplasma* spp isolation and poor semen quality of ovine

Seminal alterations	Positive (%)	OD ^a	CI ^b /95%
↓ Vigor	3 (9.09)	2.00	0.33 – 11.97
↓ Motility	7 (21.21)	0.86	0.20 – 3.66
↓ Sperm concentration	6 (18.18)	2.50	0.57 – 10.94
Presence of clumps	3 (9.09)	2.00	0.33 – 11.97
Polymorphonuclear	4 (12.12)	4.75	0.72 – 31.38

^aOD=Odds Ratio, ^bCI=Confidence Interval and ↓=Low

Table 2. Estimated risk regarding to association between *Ureaplasma* spp isolation and poor semen quality of ovine

Seminal alterations	Positive (%)	OD ^a	CI ^b /95%
↓ Vigor	0	0.50	0.02 – 10.58
↓ Motility	2 (6.06)	0.61	0.07 – 4.99
↓ Sperm concentration	2 (6.06)	1.90	0.23 – 15.59
Presence of clumps	2 (6.06)	6.25	0.67 – 57.93
Polymorphonuclear	1 (3.03)	1.60	0.14 – 18.73

^aOD=Odds Ratio, ^bCI=Confidence Interval and ↓Low

Table 3. Estimated risk regarding to association between *Mycoplasma* spp and *Ureaplasma* spp isolation and poor semen quality of ovine

Seminal alterations	Positive (%)	OD ^a	CI ^b /95%
↓ Vigor	0	0.54	0.02 – 11.80
↓ Motility	2 (6.06)	1.33	0.11 – 16.40
↓ Sperm concentration	1 (3.03)	0.86	0.07 – 10.66
Presence of clumps	2 (6.06)	13.0	0.94 – 178.88
Polymorphonuclear cells	1 (3.03)	2.50	0.19 – 33.19

^aOD=Odds Ratio, ^bCI=Confidence Interval and ↓ Low

4. Discussion

Mycoplasma isolation from ovine semen was not

reported yet in Brazil. Although it was studied 33 samples the obtained frequencies of 36.36% for *Mycoplasma* spp. and 12.12% for *Ureaplasma* spp. are the first national reference and points that the percentage of this infection is probably higher.

Isolation of mycoplasmas in ovine is not usually performed in other countries. Kappor *et al.* [11], in India, *Mycoplasma* spp. and *Acholeplasma* spp. were isolated in 17.6% from 68 semen samples. Trichard *et al.* [20], in South Africa found mycoplasmas in 83% of samples from ovine with ulcerative balanoposthitis and vulvovaginitis and 36% in healthy animals. Nicholas *et al.* [10], in Great Britain reported the isolation of *Mycoplasma* serogroup 11 in vaginal mucus from 23 female infertile sheep. It was showed the interference of these microorganisms in ovine breeding.

Contamination of semen and preputial mucus with *U. diversum* was firstly mentioned in 1969 in England, when ten preputial cultures and 84% in 32 samples of fresh semen were positive for the agent. [40] ONOVIRAN *et al.*, (1975), in Canada observed 132(35%) positive cultures for *U. diversum* in preputial mucous, 140(24%) in fresh semen samples and 42(14%) in processed semen. In 1978, Czechoslovakia, this ureaplasma was recovered from 202 samples of bull semen in insemination centers. [41]

The highest rate of positive cultures for *Mycoplasma* (71%) was obtained in cows with low fertility compared with 24% of cows culled for other reasons. [42]

FISH *et al.* (1985), Canada, reported that 28% of bulls used for artificial insemination had semen contaminated with species of *Mycoplasma* and *M. bovis* was not recovered. GARCIA *et al.* (1986), in the same country, studied 2950 samples of semen and *M. bovis* was. Ball *et al.* (1987) [43] isolated *Mycoplasma* spp in 46% of 332 samples of fresh semen of bulls. In 32% of these positive samples, were also infected with *U. diversum*.

LE GRAND *et al.* (1995) [44], in France, isolated *U. diversum* in 74% (37/50) of semen samples. Serogroups B and C were predominant in males.

M. bovis was the first bovine mycoplasma identified in Brazil by ROSSINI, (1978) [45] in calves with pneumonia in a property located in State of São Paulo. Subsequently, LIBERAL *et al.* (1982) [46] reported the isolation of *Mycoplasma* spp. in cases of bovine pneumonia in the State of Rio de Janeiro.

The mycoplasma transmission through the infected semen justify in part the diversity of findings. Lingwood *et al.* [21] and Quinn *et al.* [22] showed the adherence of *U. diversum* to bovine spermatozoa through sulfoglycolipids. Eaglesome and Garcia [22] described the interference of *M. bovis* in the fertilization processes. The presented data and the diversity of the host-parasite relationship in mycoplasma strongly suggest a better control of these bacteria in ovine breeding or rising.

The motility and other parameters of ovine spermatozoa analysed in present study were influenced by mycoplasma infection. It was possible to estimate a risk of 4.75 higher to

found these bacteria in polymorphonuclear-PMN cells in infected ovine semen (Table. 1). When the semen is infected with *Ureaplasma* spp the estimative risk to produce semen clots was 6,25 higher if compared with mycoplasma free semen. It was detected an OR>1 for a lactescent appearance, spermatozoa density and PMN (Table 2). The same OR and semen parameters was obtained for the co-infection of *Mycoplasma* spp and *Ureaplasma* spp. In this study the estimated risk was 1,33, 13, 2,5 times higher for alterations of spermatozoa motility, clots and PMN in semen respectively (Table. 3).

Cardoso *et al.* [24], studied bovine semen infected with *Mycoplasma* spp and obtained a risk of 1.02 higher for the lactescent semen and 2.7 higher for spermatozoa density. In presence of *U. diversum* the risk was 1.32 higher for clumping. In the present study it was detected a higher risk in ovine semen quality when infected with mycoplasma than described for bovines. Nicholas *et al.* [10] mentioned a decreasing motility of bovine spermatozoa and low fertility in reproductive cattle infected with *Mycoplasma* serogroup 11. Animals with orchitis presented a degeneration of spermatozoa tails. Ak *et al.* [12], experimentally inoculated *M. agalactiae* in bovine semen and mentioned a decreasing volume, motility and density of spermatozoa and an increase of abnormal spermatozoa.

In 1977 Jurmanova and Sterbova associated a low sperm motility (60%) and the semen contamination by mycoplasma and ureaplasma adhered on these cells. It was observed by a reduced motility (60%) and [26].

There are infectious agents more commonly associated with animal reproductive disorders: *Brucella* spp, *Campylobacter* spp, *Letospira* spp. [28] [35] However, many other are less mentioned, as Mollicutes, listed in schedule B of the OIE, and therefore are extremely important and should be studied. [39]

Eaglesome (1980) studies found that *M. bovis* interfered extensively in various stages of fertilization *in vitro*, first causing the decrease in the percentage of sperm capacitation, fusion with the oocyte and interfering with the organization of the pronucleus after chromatin decondensation, without, however, cause changes in their viability and motility.

5. Conclusions

It was possible to found estimate risk of 4.75 higher in polymorphonuclear-PMN cells in infected mycoplasma ovine semen and 6,25 higher estimate risk semen clots in semen infected with *Ureaplasma* spp. In this study the estimated risk was 1,33, 13, 2,5 times higher for alterations of spermatozoa motility, clots and PMN in infected semen respectively. The monitoring of mycoplasma infection in ovine production must be included in routine analysis.

REFERENCES

- [1] Shepard, M. C., Lunceford, C. D., Ford, D. K., Purcell, R. H., Taylor-Robinson, D., Razin, S. and Black, F. T., 1974, *Ureaplasma urealyticum* gen. nov. sp. nov.: proposed nomenclature for the human T (T-strain) mycoplasmas. *International J Systematic Bacteriology*, 24,160-171.
- [2] Ruhnke, H. L., Doig, P. A., Mackay, A. L., Gagnon, A. and Kierstead, M., 1978, Isolation of *Ureaplasma* from bovine granular vulvitis. *Can Vet J.*, 42, 151-155.
- [3] Doig, P. A., Ruhnke, H. L., Mackay, A. L. and Palmer, N. C., 1979, Bovine granular vulvitis associated with *Ureaplasma* infection. *Can Vet J.*, 20, 89-94.
- [4] Reid, S. W., Madill, D. G. and Vreugdenhtl, A. H., 1989, *Ureaplasma* vulvovaginitis and infertility in eight southern Ontario dairy herds. *Can Vet J.*, 30, 255.
- [5] Ruhnke, H. L. and Rosendal, S., 1994, Useful Protocols for Diagnosis of Animal Mycoplasmas. *In: Whitford, H.W.; Rosenbusch, R.F.; Lauerman, L.H. (Eds.). Mycoplasmosis in Animals: Laboratory Diagnosis.* Iowa State University Press, Ames, Iowa, 141-144.
- [6] Sanderson, M. W. and Chenoweth, P. J., 1999, The role of *Ureaplasma diversum* in bovine reproduction. *Compend Contin Educ Pract Vet*, 21, 98-111.
- [7] Ball, H. J., McCaughey, W. J., Kennedy, S. and McLoughlin, M., 1985, Experimental intrauterine inoculation of pregnant ewes with ureaplasmas. *Vet Res Com.*, 9, 35-43.
- [8] Kotani, H., Nagatomo, H. and Ogata, M., 1980, Isolation and serological comparison of ureaplasmas from goats and sheep. *Japanese J Vet.*, 42, 31-40.
- [9] Jones, G. E., Rae, A.G., Holmes, R. G., Lister, S. A., Jones, J. M. W., Grater, G. S. and Richards, N., 1983, Isolation of exotic mycoplasmas from sheep in England. *Vet Rec.*, 3, 540.
- [10] Nicholas, R. A. J., Wessels, M., Orme, P. K., Wood, E. and Sachse, K., 1999, Isolation of *Mycoplasma ovine/caprino* serogroup 11 from infertile sheep in Britain. *Vet Rec.*, 9, 434.
- [11] Kapoor, S. G., Singh, P. P. and Pathak, R. C., 1984, Prevalence of mycoplasma/acholeplasma in the genital tract of sheep. *Indian J Animal Sci.*, 54 (7), 553-556.
- [12] Ak, K., Ak, S., Gurel, A., Hasoksuz, M., Baran, A., Özturkler, Y., Ileri, I. K. and Minbay, A., 1995, Experimental studies on the effects of *Mycoplasma agalactiae* on spermatological characters and genitals in rams. *Pendik Vet Mikrobiyol Derg.*, 26, 2.
- [13] Fe, C., Amore, J., Martin, A. G., Sánchez A., Contreras, A. and Corrales, J. C., 2009, *Mycoplasma agalactiae* detected in the semen of goat bucks, *Theriogenology*, 72 (9), 1278-1281.
- [14] Livingston Junior, C. W., Gauer, B. B. and Shelton, M. A., 1978, Specific *Ureaplasma* serotype associated with ovine uterine infections. *Am J Vet Res.*, 39 (10), 1699-1701.
- [15] Johansson, K. L. and Pettersson, B., 2002, Taxonomy of *Mollicutes*; *In: RAZIN, S., HERMANN, R. (Eds). Molecular Biology and Pathogenicity of Mycoplasmas.* New York: Kluwer Academic/ Plenum Publishers, 1-29.
- [16] Howard, C. J., Pocock, D. H. and Gourlay, R. N., 1978, Base composition of deoxyribonucleic acid from ureaplasmas isolated from various animal species. *International J of System Bacteriol*, 28, 599-601.
- [17] Howard, C. J. and Pocock, D. H., 1983, Comparison of *Ureaplasmas* from sheep and goats with *Ureaplasma diversum* and *U. urealyticum*. *J Gen Microbiol.*, 129, 3197-3202.
- [18] Tully, J. G., 1995, Culture medium formulation for primary isolation and maintenance of mollicutes. *In: Tully, J.G. & Razin, S. (Eds.). Molecular and Diagnostic Procedures in Mycoplasmaology*, v.1, Academic Press, Inc. San Diego, California, 33-40.
- [19] Colégio Brasileiro de Reprodução Animal, 1998, Manual para exame andrológico e avaliação de sêmen animal. 2.ed. Belo Horizonte.
- [20] Trichard, C. J. V., Jordaan, P., Prozesky, L., Jacobsz, E. P., Henton, M. M. H., 1993, The identification of *Mycoplasma mycoides mycoides* LC as aetiological agent of balanoposthitis and vulvovaginitis in sheep in South Africa. *Onderstepoort J. Vet. Res.*, 60, 29-37.
- [21] Lingwood, C. A., Quinn, P. A., Wilansky, S., Nutikka, A., Ruhnke, H. L. and Miller, R. B., 1990, Common sulfoglycolipid receptor for mycoplasmas involved in animal end human infertility. *Biol Reprod.*, 43, 440-443.
- [22] Eaglesome, M. D. and Garcia, M. M., 1990, The effect of *Mycoplasma bovis* on fertilization processes in vitro with bull spermatozoa and zone-free hamster oocytes. *Vet Microbiol.*, 21, 329-337.
- [23] Quinn, P. A. and Fortier, M. A. Growth and attachment of *Ureaplasma diversum* in co-culture with bovine endometrial cells. *In: Conference of International Organization for Mycoplasmaology*, 10th, 1994, Toulouse, Fr. Proceedings, Fr:1994, p.337. Abstract.
- [24] Cardoso, M. V., Gregory, L., Teixeira, S. R., Scarcelli, E., Brandão, F. Z. and Genovez, M. E. Alterações na qualidade seminal associadas à *Mycoplasma* spp e *Ureaplasma diversum* em sêmen bovino "in natura". *Rev. Bras. Reprod. Anim.*, 25 (2), 240-242.
- [25] Albertsen, B. E. Pleuropneumonia-like organisms in the semen of Danish artificial insemination bulls. *Nordish Veterinaermedicin*, v. 7, p. 169-201, 1955.
- [26] Jurmanova, K.; Sterbova, J. Correlation between impaired spermatozoan motility and mycoplasma finding in bull semen. *The Veterinary Record*. V. 100, p. 157-158, 1997.
- [27] Pilazec, J.; Truscynski, m. Affinity of microorganisms of the genus *Ureaplasma* to the reproductive organs of cattle. *Comparative Immunology, microbiology and infectious diseases*, v. 11, p. 177-180, 1988.
- [28] Eaglesome, m. D.; Garcia, M., M.; Stewart, R., B., Microbial agents associated with bovine genital tract infections and semen. Part II. *Haemophilus sonnus*, *Mycoplasma* spp and *Ureaplasma* spp. *Chlamydia; Pathogens and semen contaminants; treatment of bull semen with antimicrobial agents.* *The Veterinary Bulletin*, v. 62, p. 887-910, 1992.
- [29] Panangala, V. S.; Winter, A. J.; Wijesinha, A.; Foote, R. H., Decreased motility of Bull spermatozoa caused by *Mycoplasma bonigenitalium*. *American journal of Veterinary Research*, v. 42, n. 12, p. 2090-2093, 1981.

- [30] Doig, P. A.; Ruhnke, H. L., *Ureaplasma* (T strain mycoplasma) infection in the bovine reproductive tract. American Association of Bovine Practitioners Proceedings, v. 13, p.127-136, 1981b.
- [31] Fish, N. A., Rosendal, S.; Miller, R. B., The distribution of Mycoplasmas and ureaplasmas in the genital tract of normal artificial insemination bulls. The Canadian Veterinary Journal, v. 26, p.13-15, 1985.
- [32] Hall, c. E.; Mcentee, K. reduced post-thawing survival of sperm in bull with Mycoplasmal vesiculitis (Brief Communication). The Cornell Veterinarian, v.71, p. 111-2, 1981.
- [33] Huffman, E. M.; Christensen, V., Hird, D.; Jasper, D., Epidemiology of bovine genital ureaplasma infection. Proceedings of the Society of Theriogenology. P. 67-71, 1985.
- [34] Jasper, D. E., Bovine mastitis due to mycoplasma. Revue Scientifique et Technique (Internation Office of Epizootics). V. 6, p.801-807, 1987.
- [35] Kirkbride, C. A. Mycoplasma, Ureaplasma and Acholeplasma infections of bovine genitalia. The Veterinary clinics of north America. Food Animal Practice, v. 3, p. 575-591, 1987.
- [36] Rae, D. O.; Chenoweth, P. J. Brown, m. B. *Ureaplasma* infection in the bovine. Archives of STD/HIV Research, v.7, p. 239-243, 1995.
- [37] Britton, A. P., Miller, R. B.; Ruuhnke, H. L.; Johnson, W. H. The recovery of Ureaplasmas from bovine embryos following *in vitro* exposure and ten washes. Theriogenology. V. 30, p. 997-1003, 1988.
- [38] Miller, r. B.; Chelmonska-soyta, A.; Smits, B.; Foster, R.; rosendal, S. *Ureaplasma diversum* as a cause of reproductive disease in cattle. The Veterinary clinics of North America. Food Animal Practice. V.10, p. 479-490. 1994.
- [39] Thbier, M.; Guerin, B. Hygienic aspects of storage and use of semen for artificial insemination. Animal Reproduction Science, v.62, p. 233-251, 2000.
- [40] Taylor-Robinson, D.; Thomas, M.; Dawson, P. L. The isolation of T-mycoplasmas from the urogenital tract of bulls. J. Med. Microbiol., v.2, p.527-533, 1969.
- [41] DOIG, P.A. Bovine genital mycoplasmosis. Can. Vet. J., v. 22,p.339-343, 1981a.
- [42] GOURLAY, R. N. Significance of Mycoplasma infection in cattle. J. Am. Vet. Med. Assoc., v. 163, n. 7, p. 905 - 909, 1973.
- [43] BALL, H.J.; LOGAN, E.F.; ORR, W. Isolation of mycoplasma from bovine semen in Northern Ireland. Vet. Rec.,v.121, p.322-324, 1987.
- [44] LE GRAND, D.; POUMARAT, F.; MARTEL, J.L. Infectious genital disease by Ureaplasma diversum, investigations on bovine in France. Vet. Res., v.26, n.1, p.11-20, 1995.
- [45] ROSSINI, A.J. Contribuição ao estudo de micoplasmore bovina: isolamento de Mycoplasma bovis em bezerros acometidos de pneumonia . São Paulo: 1978. 49p. [Dissertação (Mestrado) - Instituto de Ciências Biomédicas, Universidade de São Paulo, São Paulo].
- [46] LIBERAL, M.H.T.; ROMINJN, P.C.; VOLLU, E.W. Presença de Mycoplasma spp. em pulmão de bezerro de até um ano de idade. Comun. Téc. PESAGRO, Rio de Janeiro, v.8, n.1/3, 1982