PERCENTAGE OF DOGS SEROPOSITIVE FOR *Brucella canis* WITH REPRODUCTIVE PROBLEMS TREATED AT THE VETERINARY HOSPITAL OF UNIVERSIDADE ESTATUDAL DE LONDRINA (UEL)

PORCENTAGEM DE CÃES SOROPOSITIVOS PARA Brucella canis APRESENTANDO PROBLEMAS REPRODUTIVOS ATENDIDOS NO HOSPITAL VETERINÁRIO DA UNIVERSIDADE ESTADUAL DE LONDRINA

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SUMMARY

Canine Brucellosis is an infectious disease, whose main agent is *Brucella canis.* It is mainly characterized by miscarriage and sterility in females and orchitis and epididymitis in males. It is a worldwide distributed zoonotic disease. This study aimed to investigate the occurrence of *Brucella canis* antibodies in animals with reproductive problems seen at Veterinary Hospital of the State University of Londrina and to evaluate the characteristics of the animals studied. A total of 22 blood samples were collected from male and female mixed breed dogs of different breeds and ages. These samples were stored and evaluated by serologic testing in agarose gel immunodiffusion (AGID). The seropositivity was 4.54%. It was concluded that despite the low sampling and low seropositivity, *Brucella canis* infection is present in dogs in Londrina and further studies should be conducted to adopt prevention and control measures to prevent the spread of the agent.

KEY-WORDS: *Brucella canis.*Serology. Reproductive problems.

RESUMO

A brucelose canina é uma doença infectocontagiosa, tendo como principal agente a *Brucella canis*. É caracterizada principalmente por abortamentos e esterilidade nas fêmeas, e orquite e epididimite nos machos. Possui caráter zoonótico e está mundialmente distribuída. Este estudo teve como objetivo investigar a ocorrência de anticorpos anti-*Brucella canis* em animais que apresentavam problemas reprodutivos e avaliar as características dos animais estudados, atendidos no Hospital Veterinário da Universidade Estadual de Londrina. Foram colhidas 22 amostras sanguíneas de cães machos e fêmeas, de idades, raças variadas, e sem raça definida. Estas amostras foram armazenadas e avaliadas pelo teste sorológico de imunodifusão em gel de agarose (IDGA). A soropositividade encontrada foi de 4,54%. Concluiu-se que apesar da baixa amostragem e baixa soropositividade, a infecção por *Brucella canis* ocorre em cães da cidade de Londrina e devem ser empregados estudos posteriores na cidade, bem como medidas de controle e profilaxia para evitar a disseminação do agente.

PALAVRAS-CHAVE: *Brucella canis.* Sorologia. Problemas reprodutivos.

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INTRODUCTION

Canine brucellosis is a chronic infectious zoonotic disease whose main etiological agent, the *Brucella canis* bacterium, infects domestic and wild canids, and humans, as well (CARMICHAEL & GRENEE, 2006).

It has worldwide distribution with variable prevalence depending on the region, diagnosis method and sample numbers. It is of great economic and social importance, because it is widespread in large cities, leading to public health risks and large economic losses in commercial kennels (POESTER et al., 2002; ACHA & SZYFRES, 2001; VARGAS et al., 1996).

It is transmitted directly by microorganisms that enter the mucosa (SUZUKI et al., 2008). The bacteremia caused lasts long periods with predominant reproductive symptoms. In females, miscarriages occur mainly at the end of gestation. In males, it is characterized by epididymitis, prostatitis, testicular atrophy and infertility. In humans, the main clinical signs are similar to the flu (AZEVEDO et al., 2003; GRENEE & CARMICHAEL, 2006).

The diagnosis is performed by isolating the bacterium or serological tests (AZEVEDO et al., 2004; FERREIRA et al., 2003). The most commonly used serological test is the agarose gel immunodiffusion (AGID) because it is practical, fast, and can analyze a large number of samples. In addition, it can detect chronic infections since antibodies can be detected from 8 to 12 weeks and even years after infection (FERREIRA et al., 2003; AZEVEDO et al., 2004; MINHARRO et al., 2005).

The recovery of the infected animal may occur spontaneously, while the treatment can accelerate recovery (GRENEE & CARMICHAEL, 2006). The treatment is generally based on antibiotic therapy, but the results are uncertain and recurrences are common (WANKE, 2004). Other indications are also to sterilize infected animals, to treat other affected organs, and to disinfect the environment with iodophor or quaternary ammonium (CARMICHAEL & GRENEE, 2006).

The objective of this study is to determine the percentage of animals positive for *anti-Brucella canis* antibodies due to its relevance to public health, economic importance for dog breeders and the absence of epidemiological studies on the prevalence of this agent in the canine population seen at the Veterinary Hospital of Londrina State University with reproductive problems.

MATERIAL AND METHODS

The study was conducted at the Veterinary Hospital of the University with dogs seen at the Small Animal Theriogenology Service (TAC) and Emergency Room (ER) with reproductive problems. Serological analyses were conducted at the Laboratory of Veterinary Microbiology and Infectious Diseases of the Department of Veterinary Preventive Medicine of the State University of Londrina (UEL).

Between January and April 2013, blood samples were collected from 22 male and female dogs, of varying ages and breeds that were seen at the hospital due to historical and/or clinical signs indicating reproductive problems. The samples were collected by venipuncture of the jugular vein using a 5-ml syringe and 25mm x 0.7mm needle in vacuum tubes without anticoagulant (dry tube).

The samples were sent to the Laboratory of Veterinary Microbiology, and after clot retraction and blood separation, the serum was stored at -20°C in plastic microtubes until the agarose gel immunodiffusion (AGID) test was performed. The antigen kit for diagnosis of *Brucella ovis* produced by the Institute of Technology of Paraná State (TECPAR), which uses the lipopolysaccharide and protein antigen of *Brucella ovis*, was used.

The agar gel was prepared and distributed into glass blades, with seven wells, one central and six peripheral. Positive control serum interleaved with test serum samples were immediately placed in the peripheral wells while the antigen was placed in the center well. The slides were placed in a humid box and incubated at room temperature. Readings were performed after 24 and 48 hours using indirect lighting system and black background for better viewing; the final result was the reading after 48 hours. Positive samples were those whose precipitation line showed identification with the line formed by the control serum. The negative samples formed no precipitation line or the formed line showed no identification with the control.

Alongside the tests, the owners of the dogs were asked to answer an epidemiological questionnaire to evaluate the profile of the analyzed animals and compare risk factors already studied in other works. The variables were: area of origin, race, gender, age, reproductive problems and vaccination history.

NOTE – Despite being informed about the study, this study was not submitted to the ethics committee because the animals used were already undergoing animal care and exams at the Veterinary Hospital.

RESULTS AND DISCUSSION

The results of the AGID test showed that of the total 22 serum samples evaluated only one was reactive, resulting in 4.5% seropositivity for the presence of *Brucella canis* in dogs seen with reproductive problems at the Veterinary Hospital of UEL.

Similar results have been reported by Germano et al. (1987) in Campinas, SP, of 5.4%; Souza (2001) in Belo Horizonte, MG, 4.8%; Aguiar et al. (2005) in Monte Negro, 3.6%; Cavalcanti et al. (2006) in the metropolitan region of Salvador, BA, 5.8%; Bezerra et al. (2012) in the region of Ilheus-Itabuna, BA, 3.4%; and, Silva et al. (2012) in the Northern region of Paraná, 4%. All these studies were performed using the

agarose gel immunodiffusion (AGID) test, but with different sampling methodologies.

More significant prevalence values were reported by Vargas et al (1996) for a kennel in Uruguaiana, RS, of 72.7%; Maia et al (1999) in Niterói, RJ, 25.7%; and Dorneles et al. (2011) in Araguaiana, TO, found 44.5%. Furthermore, less significant values were also reported by Moraes et al. (2002) in the micro-region of Botucatu Mountains, SP, a prevalence of 0.84% and Dos Reis et al. (2008) found 0.80% prevalence for homeless dogs of Sao Joao da Boa Vista, SP.

These variations in the prevalence rates can be probably explained by the different serological methods used, which have different sensitivity and specificity, the sample type, as well as the animals early infection stages when the antibodies cannot be detected depending on the diagnostic method used. In addition, some studies were conducted in commercial kennels with animals that had a history of infertility, miscarriage and stillbirth, an environment conducive to the rapid spread of infection leading to a higher prevalence (CARMICHAEL & GREENE, 2006; NICOLETTI & CHASE, 1988)

The responses to the epidemiological questionnaire allowed defining the profile of the studied animals. The following variables were determined: sex, reproductive problems, place of origin, race, age, access to the street and vaccination history. It is noteworthy that when working with epidemiological questionnaire, the results depend on the truthfulness of the responses given by the owner.

Of the animals tested, 72.7% were female (16/22) and 27.2% male (6/22) (Chart 1). Porto et al. (2008) and Castro (2012) reported no statistically significant gender predisposition for higher prevalence in either sex. The higher prevalence in females in this study can be explained by the larger number of females seen with reproductive failure at the HV - UEL. The main reproductive problems encountered in this study were pyometra 40.9% (10/22), abortion 31.9% (7/22), orchitis 18.1% (4/22), epididymitis 4.5% (1/22) and prostatitis 4.5% (1/22) (Chart 2). Megid et al. (1999) and Porto et al. (2008) reported positive animals with the same clinical signs. Porto et al. (2008) demonstrated that animals with reproductive clinical signs are approximately four times more likely to be positive compared to clinically healthy animals and reported a statistical correlation between reproductive problems and positive AGID in male dogs. Castro (2012) found a statistical association between abortion and seropositivity to *B. canis.* The prevalence of reproductive symptoms can also be explained by the larger sample of certain diseases treated at HV - UEL such as abortions and pyometra compared to the number of epididymitis and orchitis cases.

In this study, 90.9% of the animals were from urban areas (20/22) and 9.1% rural (2/22) (Chart 3). Moraes et al. (2002) and Bezerra et al. (2012) reported no significant difference for the occurrence of the disease with respect to animal origin. The highest prevalence of positive animals from urban areas can be explained by the fact that most of the samples were taken from animals that lived in urban areas.

The breeds of animals analyzed in this study were: 50% mongrel (non-defined breed, NDB) (11/22), 13.6% Pit-bull (3/22), 9% Boxer (2/22), 9% Blue Heeler (2/22), 9% Poodle (2/22), 4.5% Belgian shepherd (1/22) and 4.5% Pinscher (1/22) (Figure 4). In the first descriptions of dogs infected with *B. canis,* Beagle dogs were considered the most likely to get infected (MOORE & KAKUK, 1969). However, lately the disease has been diagnosed in dogs of various breeds (CARMICHAEL & KENNEY, 1968). This study did not evaluate any Beagle. Souza (2001), Azevedo et al. (2003) and Bezerra et al. (2012) found no statistical relationship between race and seropositive animals, showing no race bias. Cavalcanti et al. (2006) found a higher prevalence among mongrel dogs, as in the present study.

Animal ages ranged from two to thirteen years old. According to the literature, the greatest frequency of seropositive dogs is reported for one year old dogs or those of reproductive age (MAIA et al., 1999). Cavalcanti et al. (2006) found no statistically significant correlation between age and frequency of animals seropositive to *B. canis,* but the positive reaction was observed only in animals over two years old. On the other hand, Souza (2001) and Azevedo et al. (2003) found no statistical significance between age and positivity for AGID. Sexual maturity and subsequent mating, as well as the greater possibility of contact between infected animals, can help to spread the disease. Furthermore, prepubertal animals can also acquire the infection but usually the only clinical manifestation is unilateral or bilateral lymphadenopathy while for animals that have reached puberty the clinical signs are reproductive problems (CARMICHAEL & GRENEE, 2006; AZEVEDO et al., 2003).

The animals of the present study had reproductive problems and, therefore, had reached sexual maturity. This may justify the minimum age found to be two years old.

According to questionnaire responses, 77.2% of the evaluated animals had access to the street (17/22) (Table 1). Azevedo et al. (2003) reported statistically significant difference for seropositivity in animals that have unrestricted access to the street, showing this to be a risk factor since they may come into contact with other animals, thus increasing the chances of infection (CARMICHAEL & GREENE, 2006). However, other studies found no relationship between the type of semi-domiciled management and access to the street as risk factors, such as Silva et al. (2012) and Castro (2012). This study demonstrates that most of the analyzed animals with reproductive problems had access to the street.

As for vaccination history (Table 1), it was observed that 45.4% of animals (10/22) have a history of rabies vaccination and multivalent vaccine. Castro (2012) also reported vaccination rate of 54.3%. The vaccination rate in this study is quite high, taking into account that pet owners visiting the Veterinary

Hospital of the UEL have low purchasing power and it is assumed that many of them do not have the habit of vaccinating their pets or are unaware of the need for vaccination.

The animal positive to the AGID test in this study was a Boxer female, 5 years old, unvaccinated, from the urban area, with street access and contact with stray animals. The reproductive failure consisted of fetal death and miscarriage in the last third of gestation. This animal presented the risk factors of infected animals, such as miscarriage, which is the main clinical sign of brucellosis by *B. canis* in bitches (CARMICHAEL & GREENE, 2006; WANKE, 2004). The bitch was sexually mature, had access to the street and contact with stray dogs, which increased the chance of infection (CARMICHAEL & GRENEE, 2006; AZEVEDO et al., 2003).

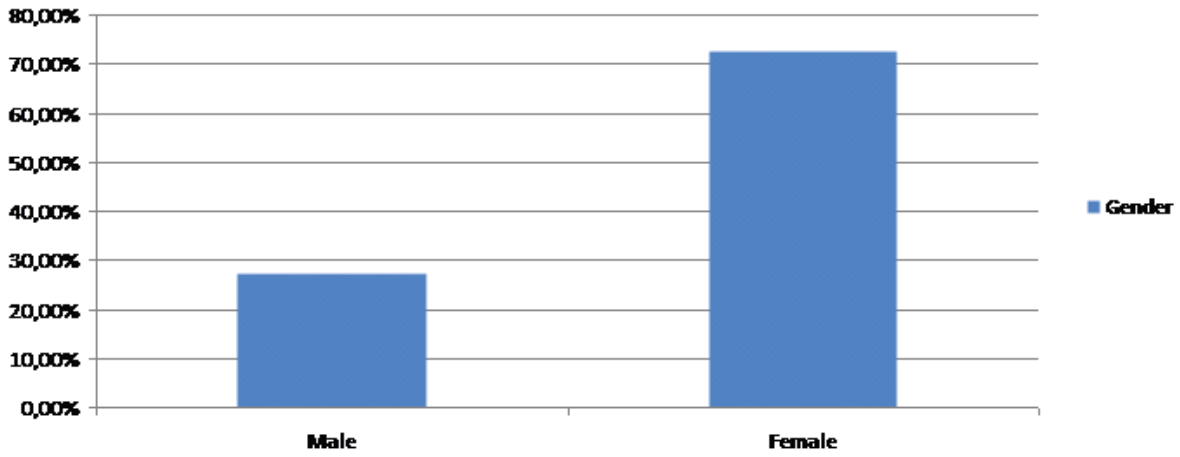

Figure 1 – Sex of the 22 dogs seen for reproductive problems at the Veterinary Hospital of the State University of Londrina, UEL, 2013.

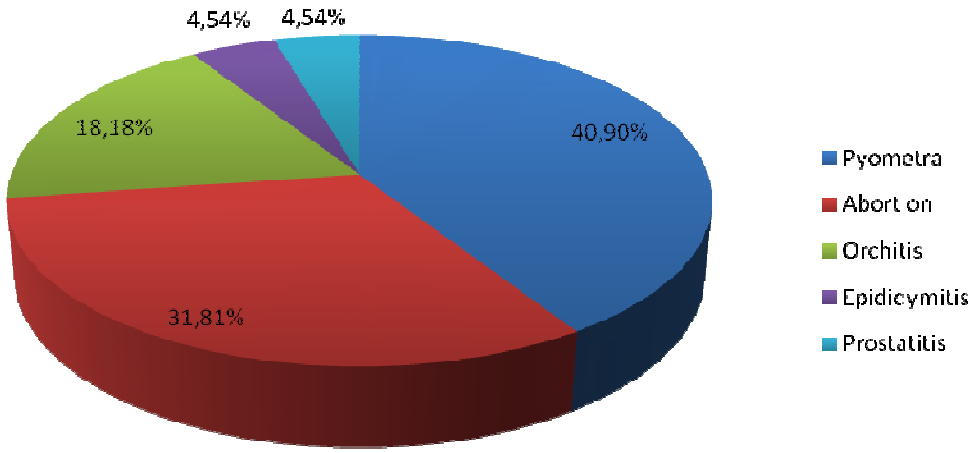

Figure 2 - Reproductive problems of the 22 dogs evaluated at the Veterinary Hospital of the UEL, 2013

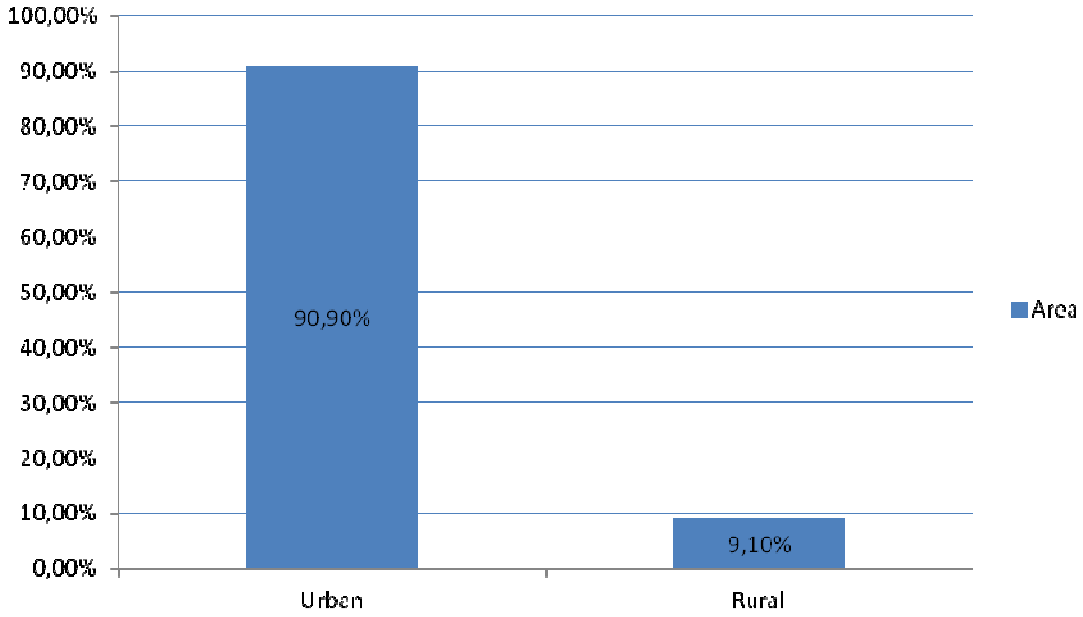

Figure 3 – Area of origin of the 22 dogs evaluated at the Veterinary Hospital of the UEL, 2013.

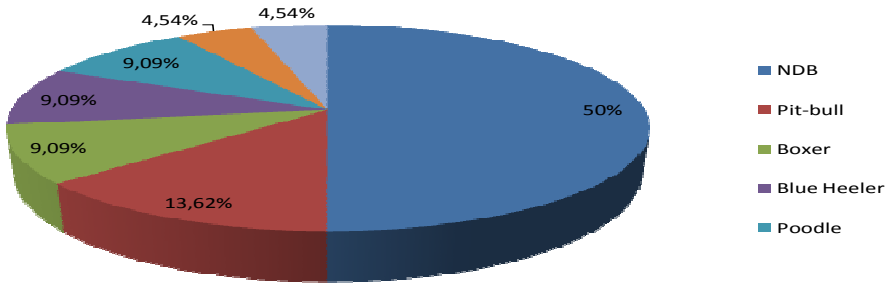

Figure 4 – Breeds of the 22 dogs with reproductive problems evaluated at the Veterinary Hospital of UEL, 2013

Table 1 – Percentage of dogs with access to the street and vaccination history of the 22 dogs seen for reproductive problems at the Veterinary Hospital of UEL, 2013.

	Access to the street	Vaccination
Yes	77.27%	45.45%
No	22.73%	54.55%

CONCLUSION

According to the results it was concluded that seropositivity for *Brucella canis* antibodies was low in animals treated at the Veterinary Hospital of UEL. Although the total number of animals evaluated is not expressive to represent the canine population of the region, the percentage of 4.5% should be considered important because it means that a portion of the canine population with reproductive problems, referred to the Veterinary Hospital, may be playing the role of *Brucella canis* reservoir, and that *B. canis* is circulating in the region exposing to infection risk not only other dogs, but humans as well. Thus, sanitary measures should be adopted to control and prevent the spread of the disease. Further studies using a representative sample of the canine population from Londrina are necessary.

REFERENCES

ACHA, P. N.; SZYFRES, B**. Zoonosis y enfermedades transmisible comunes al hombre y a los animales brucellosis**. 3.ed. Washington: OPS/OMS. p.28-56, 2001

AGUIAR, D. M.; CAVALCANTE, G. T.; VASCONCELLOS, S. A.; MEGID, J.; SALGADO, V. R.; CRUZ, T. F.; LABRUNA, M. B.; PINTER, A.; SILVA, J. C. R.; MORAES, Z. M.; CAMARGO, L. M. A.; GENNARI, S. M. Ocorrência de anticorpos anti-*Brucella abortus* e anti-*Brucella canis* em cães rurais e urbanos do Município de Monte Negro, Rondônia, Brasil. **Ciência Rural, Santa Maria**, v.35, n.5, p.1216-1219, 2005.

ALMEIDA, A. C.; SANTORELLI, A.; BRUZADELLI R. M. Z.; OLIVEIRA, M. M. N. F. Soroepidemiologia da brucelose canina causada por Brucella canis e Brucella abortus na cidade de alfenas, MG. **Arquivo Brasileiro de Medicina Veterináriae Zootecnia**, v.56, n.2, p.275-276, 2004. Disponível em:<http://www.scielo.br/pdf/abmvz/v56n2/20341.pdf >. Acesso em: 16 Out. 2012.

AZEVEDO, S. S.; BATISTA, C. S. A.; ALVES, C. J.; CLAMENTINO, I. J. Ocorrência de anticorpos anti *Brucella abortus* em cães errantes da cidade de Patos, estado da Paraíba, Brasil. **Arquivo do Institudo de Biologia,** v.70, n.4, p.499-500, out./dez., 2003.

AZEVEDO, S. S.; VASCONCELLOS, S. A.; ALVES, C. J.; KEID, L. B.; GRASSO, L. M. P. S.; MASCOLLI, R.; PINHEIRO, S. R. Comparação de três testes sorológicos aplicados ao diagnóstico da infecção de caninos por *Brucella canis.* **Brazilian Journal of Veterinary Research and Animal Science,** v. 41, p.106-112, 2004.

AZEVEDO, S. S.; VASCONCELLOS, S. A.; ALVES, C. J.; KEID, L. B.; GRASSO, L. M. P. S.; MASCOLLI, R.; PINHEIRO, S. R. Inquérito sorológico e fatores de risco para brucelose por *Brucella canis* em cães do município de Santana de Parnaíba, estado de São Paulo. **Pesquisa Veterinária Brasileira**, v. 23, n.4, p. 156-160, 2003. Disponível em: <http://www.scielo.br/pdf/pvb/v23n4/18730.pdf>. Acesso em: 23 Abril. 2013.

BEZERRA, R. A.; MENDONÇA, C. E. D.; SICUPIRA, P. M. L.; MUNHOZ, A. D.; RIBEIRO, A. R. P.; CARLOS, R. S. A.; ALBUQUERQUE, G. R. Prevalência de anticorpos anti *Brucella canis* em cães na região de Ilhéus-Itabuna, estado da Bahia, Brasil. **Revista Brasileira de Medicina Veterinária.,** v.34, n.1, p.27-30, 2012

CARMICHAEL, L. E. Abortions in 200 beagles. **Journal of the American Veterinary Medical Association**, v.149, n.8, p.1126, 1966

CARMICHAEL, L. E. Canine brucellosis: an annoted review with selected cautionary comments. Theriogenology, v.6, n.2-3, p.105-116, 1976.

CARMICHAEL, L. E.; GREENE C. E. Canine brucellosis. In**:** GREENE C.E. **Infectious diseases of the dog and cat.** 3 ed, Philadelphia: W. B. Saunders 2006. P. 369-381.

CARMICHAEL, L. E. ; KENNEY, R. M. Canine abortion caused by *Brucella canis*. **Journal of American Veterinary Medical Association**, v.152, n.6, p.605-616, 1968.

CASTRO, V. V. Ocorrência de Brucelose canina em cães antendidos no Hospital veterinário da UFMS. 2012. 24f. Trabalho de conclusão de curso (graduação) – Universidade Federal do Mato Grosso do Sul, Campo Grande, 2012.

CAVALCANTI, L. A.; DASSO, M. G.; OLIVEIRA, F. C. S.; VIEGAS, S. A. R. A.; ALMEIDA, M. G. A. R.; ANUNCIAÇÃO, A. V. M.; ALCANTARA, A. C.; BITTENCOURT, D. V. V.; OLIVEIRA, E. M. D. Pesquisa de anticorpos anti- *brucella canis* em cães provenientes da região metropolitana de Salvador. **Revista Brasileira de Saúde Produção Animal**, v.7, n.2, p.176-180, 2006. Disponível em: <http://www.rbspa.ufba.br>. ISSN 1519 9940. Acesso em: 5 jan. 2013.

DORNELES, E. M. S.; SANTOS, H.; MINHARRO,; NASCIMENTO–ROCHA, J. M.; MATHIAS, L. A.; DASSO M. G.; TIENSOLI, C. D.; HEINNEMAN, M. B.; LAGE, A. P. Anticorpos anti-*Brucella canis* e anti-*Brucella abortus* em cães de Araguaína, Tocantins. **Brazilian Journal of Veterinary Research and Animal Science**, v.48, n.2, p.167-171, 2011.

DOS REIS, C. B. M.; HOFFMANN, R. C.; SANTOS, R. S.; TURRI R. J. G.; ORIANI, M. R. G. Pesquisa de anticorpos anti-Brucella ca¬nis e anti-Brucella abortus em cães errantes da cidade de São João da Boa Vista, Estado de São Paulo, Brasil (2002- 2003). **Brazilian journal. Veterinary Reserch Animal Science**. v.45, n.1, p.32-34, 2008.

FERREIRA, T.; FIGUEIREDO, M. J.; RONCONI, M. A.; TORRES, H. M.; AQUINO, M. H. C.; GOMES, M. J. P.; SILVA, M. V.; OLIVEIRA, L. A. T. Brucelose canina: obtenção de antígenos e avaliação pela técnica de imunodifusão em gel de agarose. **Revista Brasileira de Ciência Veterinária,** v.10, n.3, p.156-160, 2003.

GERMANO, P. M. L, VASCONCELLOS, S. A. ISHIZUKA, M. M. et al. Prevalência de infecção por Brucella canis em cães da cidade de Campinas, SP, Brasil. **Revista da Faculdade de Medicina Veterinária e Zootecnia da Universidade de São Paulo**., v.24, p.27-34, 1987.

GODOY, A. M.; PERES, J. N.; BARG, L. Isolamento de Brucella canis em Minas Gerais, Brasil**. Arquivos da Escola de Medicina Veterinária da Universidade Federal de Minas Gerais**, v.29, p.35-42, 1976.

MAIA, G. R.; ROSSI, C. R. S.; ABRADIA F. Prevalência da brucelose canina nas cidades do Rio de Janeiro e Niterói-RJ. **Revista Brasileira de Reprodução Animal**., v.23, n.3, p.425-427, 1999.

MEGID, J.; BRITO, A. F.; MORAES, C. C. G.; FAVA, N.; AGOTTANI, J. Epidemiological assessment of canine brucellosis. **Arquivo Brasileiro deMedicina Veterinária e Zootecnia,** v.51, n.5, p.439-440, 1999.

MINHARRO, S.; COTTORELLO, A. C. P.; MIRANDA, K. L.; STYNEN, A. P. R.; ALVES, T. M.; LAGE, ; A. P. Diagnóstico da brucelose canina: dificuldades e estratégias. **Revista brasileira de reprodução animal,** v.29, n.3/4, p.167-173, 2005

MORAES, C. C. G.; MEGID, J; SOUZA, A. J.; CROCCI, L. C. Prevalência da brucelose canina na microrregião da serra de Botucatu, São Paulo, Brasil. **Arquivo Instituto Biológico**, v.69, n.2, p.7-10, 2002.

MOORE, J. A.; KAKUK, T. J. Male dogs naturally infected with Brucella canis. **Journal American Veterinary Medical Association**, v.155, n.8, p.1352-1358, 1969.

NICOLETTI, P.; CHASE, A. Avaliação dos métodos de diagnóstico da infecção por Brucella canis em cães. **Cães e Gatos**, p.21-23, 1988.

POESTER, F. P.; GONÇALVES, V. S. P.; LAGE, A. P. Brucellosis in Brazil. **Veterinary Microbiology**, v.90, n.1-4, p.55-62, 2002.

PORTO, W. J. N.; JUNIOR, J. W. P.; MOTA, R. A. Associação entre distúrbios reprodutivos e anticorpos anti-*Brucella* sp em cães atendidos em clínicas particulares da cidade de Maceió. **Revista Brasileira de Ciências Veterinária**, v.15, n.1, p.6-9, 2008.

SILVA, L. C. S.; JUNIOR, L. A. L.; NASSAR, J. L. B.; JUNIOR, F. A. B.; HEADLEY, S. A.; OKANO, W.; KEMPER.; TRAPP, S. M. Detecção sorológica de *Brucella canis* em cães de abrigos da região Norte do Paraná. **Semina: Ciências Agrárias, Londrina**, v.33, n.6, p.2391-2396, nov./dez. 2012.a.

SILVA, C. P. A.; ALMEIDA, A. B. P. F.; GODOY, I.; ARAÚJO, A. C. P.; AGUIAR, D. M.; SOUZA, V. R. F.; NAKAZATO, L.; DUTRA, V. Detecção molecular de *Brucella canis* em cães do Município de Cuiabá, Estado de Mato Grosso. **Ciência Rural, Santa Maria. v**.42, n.6, p.1051-1056, 2012.b.

SOUZA, L. A. Prevalência de infecção por *Brucella canis* na região metropolitana de Belo Horizonte – MG, no período de dezembro de 1999 a junho de 2000. 2001. 23f. Disserteção (Mestrado em medicina veterinária) UFMG, Belo Horizonte, 2001.

SUZUKI, E. Y.; PENHA, G. A.; UEDA, F. S.; SALVARANI, R. S.; ALVES, M. L. Brucelose canina: revisão de literatura. **Revista Científica Eletrônica de Medicina Veterinária**, ano 6, n.10, p.1-4, 2008. ISSN: 1679-7353.

VARGAS, A. C.; LAZZARI, A.; DUTRA, V.; POESTER, F. Brucelose canina: relato de caso. ***Ciencia Rural***, v.26(2): p.305-308, 1996.

WANKE, M. M. Canine brucellosis. **Animal reproduction science**, v.82-83, n.1, p.195-207, 2004.