Ciência Rural, Santa Maria, v.53:12, e20220455, 2023 http://doi.org/10.1590/0103-8478cr20220455
ISSNe 1678-4596
MICROBIOLOGY

Brucella spp. detection in blood, semen and vaginal swabs in dogs from the urban area of Cuiabá/MT, Brazil

Sara Maria Dantas da Nóbrega Dias[1*] Thalita Priscila Peres Seabra da Cruz[2] Antônio Henrique Kuczmarski[1] Luciano Nakazato[2] Valeria Dutra[2] Regina Célia Rodrigues da Paz[2]

[1]Laboratório de Pesquisa em Animais de Zoológico, Universidade Federal do Mato Grosso (UFMT), 78060-900, Cuiabá, MT, Brasil. E-mail: sara.conpleta@hotmail.com. *Corresponding author.
[2]Laboratório de Microbiologia, Universidade Federal do Mato Grosso (UFMT), Cuiabá, MT, Brasil.

ABSTRACT: Brucellosis is a chronic contagious infectious zoonosis that affects the reproductive system of animals, causing economic and health losses. This study diagnosed *Brucella spp.* in commercial kennels, comparing PCR positivity in different biological samples (blood, semen, and vaginal secretion), as well as correlating these findings with reproductive indices. Hence, we analyzed dogs from kennels in the neighboring cities of Cuiabá and Várzea Grande/MT, Brazil. The reproductive histories of the animals were obtained and blood samples were collected from all animals (n=35); in addition, semen samples were collected from males (n=9) and vaginal swabs were collected from females (n=24) to perform polymerase chain reactions (PCR) for brucellosis. The findings indicated that vaginal swab PCR is an effective test to identify Brucella spp. For males, there were more positive results when testing blood samples, possibly because the male animals were at the beginning stage of infection.
Key words: PCR, infertility, reproduction, zoonosis.

Detecção de *Brucella spp.* em sangue, sêmen e swab vaginal de cães da região metropolitana de Cuiabá/MT

RESUMO: A Brucelose é uma zoonose infectocontagiosa crônica que afeta o sistema reprodutivo dos animais, gerando prejuízos econômicos e sanitários. O objetivo do presente estudo foi diagnosticar a *Brucella spp*. em canis comerciais, comparando a positividade na PCR em diferentes amostras biológicas (sangue, sêmen e secreção vaginal) e correlacionar estes achados aos índices reprodutivos. Foram analisados cães provenientes de canis no município de Cuiabá e Várzea Grande/MT. Foi realizado o levantamento do histórico reprodutivo dos animais e em seguida, foi coletado o sangue em todos os animais (n=35), sendo sêmen nos machos (n=9) e swab vaginal (n=24) nas fêmeas para realização da técnica de Reação em Cadeia pela Polimerase (PCR) para brucelose. De acordo com os resultados, conclui-se que a PCR de swab vaginal é um teste efetivo para identificar *Brucella* spp., porém em machos, verifica-se que no sangue obtivemos mais positivos, possivelmente por estarem no início da infecção.
Palavras-chave: PCR, infertilidade, reprodução, zoonoses.

INTRODUCTION

Domestic dogs have been gaining space in families since they were first domesticated, and traits such as affection and loyalty have been responsible for their inclusion in human families (MONTEIRO & MELO, 2020). Among the diseases that affect domestic dogs, brucellosis is considered one of the main zoonoses worldwide, endangering human and animal public health (ZHOU et al., 2020).

The main clinical signs in female dogs are embryonic death, abortion, fetal reabsorption, vaginal discharge, and the birth of weakened offspring (CARMICHAEL, 1966; CARMICHAEL et al., 1968; RODRIGUES et al., 2016). The main clinical signs in males are orchitis, epididymitis, prostatitis, testicular atrophy, scrotal dermatitis, and sperm defects. However, in addition to sterility, other clinical signs such as glomerulonephritis, lymph node enlargement, hepatosplenomegaly, discospondylitis, osteomyelitis, claudication, meningoencephalitis and uveitis may occur in both sexes (LEDBETTER et al., 2009; HOLST et al., 2012; RODRIGUES et al. 2016).

We can divide the species of the genus *Brucella* into two groups, smooth and rough, according to the presence of lipopolysaccharides in the bacterial cell wall, which is a determining factor for the virulence of the bacterium (CORBEL, 1997; CARDOSO et al., 2006).

Received 08.12.22 Approved 02.07.23 Returned by the author 04.03.23
CR-2022-0455.R3
Editors: Rudi Weiblen Juliana Felipetto Cargnelutti

B. canis has a worldwide distribution and has been reported in different parts of the world, such as the Americas (North, Central and South), Asia, Africa and Europe. Only New Zealand and Australia have no records of *B. canis*; however, Australia has reported some *B. suis* infections in dogs used for wild pig hunting (UEDA et al., 1974; GARDNER et al., 1997; WANKE, 2004; LUCERO et al., 2008; LEDBETTER et al., 2009; CORRENTE et al., 2010; GYURANECZ et al., 2011; CHINYOKA et al., 2014; MOR et al., 2016; WHATMORE et al., 2017; HUBBARD et al., 2018; BUHMANN et al., 2019; GALARCE et al., 2020).

In Brazil, several serological, microbiological and molecular studies have been carried out on *B. canis* in different regions of the country. For example, in São Paulo (LARSSON et al., 1984), in Rio Grande do Sul (VARGAS et al., 1996), in Pará (CARVALHO et al., 2000), in Paraíba (ALVES et al., 2003), in Minas Gerais (ALMEIDA et al., 2004), in Rondônia (AGUIAR et al., 2005), in Bahia (CAVALCANTI et al., 2006), in Rio de Janeiro (FERREIRA et al., 2007), in Tocantins (DORNELES et al., 2011), in Mato Grosso (SILVA et al., 2012), in Paraná (DREER et al., 2013), in Rio Grande do Norte (FERNANDES et al., 2013), in Mato Grosso do Sul (OLIVEIRA et al., 2019) and in Brasília (VOLKWEIS et al., 2020).

However, whether worldwide or nationally, there is difficulty in estimating the prevalence of *B. canis* due to the lack of mandatory interstate and international tests (MAPA, 2018; SANTOS et al., 2021). The World Organization for Animal Health (OIE) and the Brazilian Ministry of Agriculture, Livestock and Supply (MAPA), via the National Program for the Control and Eradication of Brucellosis and Animal Tuberculosis (PNCEBT), aim to control and cull only animals affected by ovine, swine, bovine and buffalo brucellosis. In Brazil, *B. canis* is not mandatorily reported, and a vaccine only exists for *B. abortus*; this vaccine is mandatory only for bovine and buffalo females aged between three and eight months, and one of the negative factors of the vaccine is that it has the characteristic of compromising the serological test (VARGAS et al., 1996; KEID, 2004; LAGE et al., 2006; GOMES, 2009; MAPA, 2018; OIE, 2018).

The diagnosis of canine brucellosis is still a challenge (VOLKWEIS et al., 2018), and this difficulty may be due to serological tests presenting nonspecific reactions, which may present as false-positive results. However, the PCR test has high sensitivity, thereby directly influencing diagnostic accuracy. Another advantage of PCR is the ability to test using different biological samples, such as semen and secretions.

Due to the zoonotic risk, the indication is for affected dogs to be euthanized; however, euthanasia is not mandatory. Brucellosis treatment consists of administering antibiotics and castrating the affected animal, but the treatment is long, has a small chance of cure (VOLKWEIS et al., 2018), and after treatment, it is necessary to monitor the animal (JAMES et al., 2017).

In several regions of the world, brucellosis in dogs is an endemic disease (HENSEL et al., 2018; VOLKWEIS et al., 2018); it will continue to be a problem for public health and animal welfare if it continues to be neglected and no political and public health measures are taken (MOL et al., 2019).

The present study diagnosed *Brucella spp*. in commercial kennels, comparing PCR positivity obtained with different biological samples (blood, semen and vaginal secretion) and correlating these findings with reproductive indices.

MATERIALS AND METHODS

Thirty-five domestic dogs, 24 females and 11 males, of different ages, weights and breeds were randomly selected from four commercial kennels in the cities of Cuiabá and Várzea Grande, in the state of Mato Grosso (MT), Brazil.

The following were performed: clinical evaluation, andrological and gynecological examinations, as well as a survey of the clinical and reproductive histories of the animals. For PCR (Polimerase Chain Reaction) diagnosis, blood samples were collected from all animals (n=35); in addition, semen samples were collected from males (n=9) and vaginal swabs were collected from females (n=24). All specimens were individually packaged and taken to the UFMT Microbiology laboratory, where they were kept refrigerated at -20 °C until DNA extraction.

Andrological exam

The scrotum, testicles, epididymis, penis and foreskin were inspected, followed by a consistency assessment and testicular biometry. All animals underwent semen collection through manual manipulation. Seminal evaluations consisted of volume (mL), motility (0–100%), vigor (0–5), and pH. Subsequently, the semen was diluted at a 1:20 concentration in a 10% saline formalin solution to verify the sperm concentration in a Neubauer chamber and the sperm morphology in a humid chamber, after which defects were classified as major and minor using a phase contrast microscope (CBRA, 2013).

Membrane integrity was assessed using eosin-nigrosin staining (SWANSON & BEARDEN, 1951), and acrosomal integrity was assessed using rose bengal-Fast Green staining (POPE et al., 1991) and counting 200 cells under an optical microscope. Semen samples were placed in sterile plastic tubes and sent to the UFMT Microbiology Laboratory for analysis.

Gynecological examination

Inspection of the vagina and ultrasound of the reproductive system were performed, followed by the collection of material through the introduction of a sterile vaginal swab at an angle of 45° and then 180° so that material could be collected from the most cranial region of the vagina. The sample was individually packaged and sent to the UFMT Microbiology Laboratory for analysis.

Polymerase chain reaction (PCR)

DNA extraction from biological materials was performed by bacterial lysis using 1 mL lysis buffer (100 mM NaCl, 25 mM EDTA, 100 mM Tris-HCl pH 8.0, 0.5% SDS, 0.1 mg proteinase K) under incubation and shaking at 56 °C overnight. After centrifugation, the precipitate was treated with phenol–chloroform as described by SAMBROOK & RUSSEL (2004). The DNA was resuspended in 50 µL of ultrapure water. The integrity and quality of the extracted DNA was verified by electrophoresis at 100 V for 40 min in a 1.5% agarose gel stained with Gel-Red (Biotium). Bands were visualized on ChemiDocTM XRS using ImageLabTM® software. The material was stored at -20 °C until use in molecular tests.

Molecular tests were subsequently performed by PCR, following the protocol in KIM et al. (2007), using oligonucleotides B2N-1 (GTCGCGGATTCTACCTCACCT) and B2N-2 (TAAGCAGGTAAGAGGCAATTT) that amplify a fragment of 280 base pairs (pb) for species of *Brucella spp*.

The reactions were amplified in a MyCyclerTM thermal cycler (Bio-Rad) with initial denaturation for 5 minutes at 95 °C, followed by 35 cycles of denaturation for 60 seconds at 95 °C, hybridization for 30 seconds at 55 °C, extension for 1 minute at 72 °C, concluding with a final extension cycle at 72 °C for 5 minutes.

The concentrations of the reagents were as follows: 2.5 µl of 10X reaction buffer, 2 µl of $MgCl_2$ (at a concentration of 50 mM), 0.75 µl of primer, 5 µl of DNTP, 0.2 µl of TaqDNA Polymerase (Recombinant, Invitrogen), 1 µl of DNA and ultrapure water.

The amplified products were analyzed by electrophoresis on a 1.5% agarose gel, checked by electrophoresis at 100 V for 40 min on a 1.5% agarose gel stained with Gel-Red (Biotium) and visualized on ChemiDocTM XRS using ImageLabTM® software.

Statistical analysis

The Kappa coefficient and the McNemar test were used to verify the concordance of the genetic material tests to detect *Brucella spp*. in animals. All analyses were performed considering 95% confidence and were performed using the statistical software R (R CORE TEAM, 2022).

RESULTS AND DISCUSSION

In general, no clinical signs compatible with infection, such as fever, weight loss, apathy or decreased appetite, were observed, despite the significant occurrence of *Brucella spp*. This absence of symptoms confirmed the difficulty in diagnosing brucellosis clinically (CARMICHAEL et al., 1968; KEID, 2017).

The PCR results for the different biological samples are shown in figure 1. For the tests on semen, it was not possible to collect a sample in Male 2 (M2), and Male 3 (M3) was castrated. Table 1 presents semen analyses data. Table 2 presents data on consistency, testicular biometry and sperm morphology. Ultrasound evaluations of the female reproductive system indicated an abnormality in the body of uterus of female 1 (F1).

Brucella canis infection in dogs has been described in several countries, and transmission occurs during the mating period or through contact with contaminated material. In humans, records show that most cases of infection are associated with laboratory workers and individuals working in kennels (VARGA et al., 1996).

It was not possible to track the animals that were in the kennels when the study was performed; however, 15 (63.6%) males and 7 (62.5%) females came from other kennels, which may have led to the entry of infected animals into the evaluated kennels. MOORE et al. (1970) noted that the dissemination of canine brucellosis is associated with the movement of dogs from one region to another. Another likely way of entry may be through the movement of breeding animals, mainly males that are frequently used to cover females from other properties, thereby allowing disease transmission to these dams and subsequent dissemination of canine brucellosis in their kennel of origin.

Pregnancy was confirmed in 17 (70.8%) of the females, and only 2 (8.4%) of the females showed clinical signs; F1 (4.2%) exhibited infertility and female 2 (F2) (4.2%) had an abortion at 60 days of gestation.

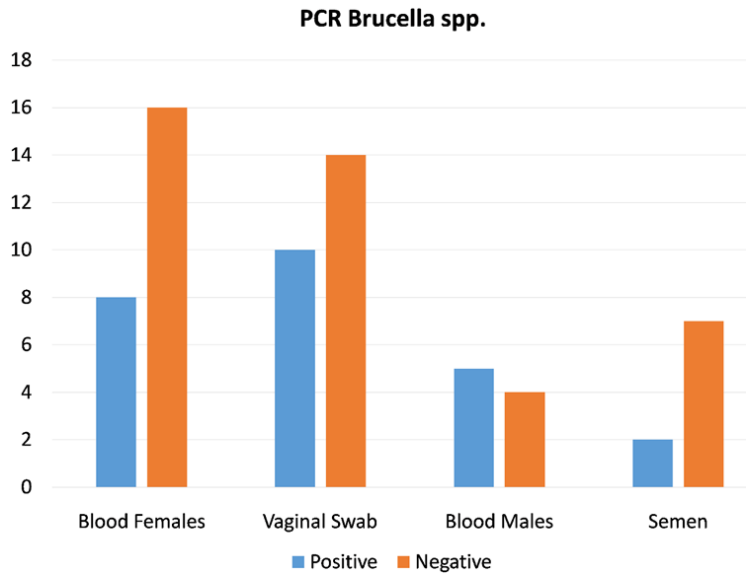

Figure 1 - PCR results for *Brucella spp.* in females (blood females and vaginal swab) and males (blood males and semen).

Both animals were negative for *Brucella spp*. according to the blood PCR and positive according to the vaginal swab PCR. Both clinical signs exhibited are typical of brucellosis in bitches (GARLACE et al., 2020).

Regarding the results of PCR tests to detect *Brucella spp*. in females, as observed in table 3, 6 (25%) animals had positive PCR results in vaginal swab samples only and 4 (16.7%) animals showed positive results in blood samples only. In addition, 4 (16.7%) females showed positive PCR results for *Brucella spp.* in both blood and vaginal swab samples, thus totaling 10 (41.7%) animals with positive PCR results for *Brucella spp*. in vaginal swab samples and 8 (33.3%) animals with positive PCR results for *Brucella spp*. in blood. These findings suggested that *Brucella spp*. can be detected through vaginal swabs in animals without clinical symptoms, thereby facilitating the diagnosis and control of infected animals on the property. In addition, another advantage of using this material would be the ease of collection, since performing a vaginal swab does not require the female to be restrained.

Table 1 - Seminal evaluation of dogs from commercial kennels in Cuiabá and Várzea Grande/MT, Brazil.

Animal	Volume (mL)	Motility (%)	Vigor (1-5)	pH	Membrane Integrity (%)	Acrosome Integrity (%)
1’’	3.3	50	3	7	58	47
4’’’	7.7	75	4	6	42	69
5’’	2.8	70	3	6	55	66
6’’	17.1	80	4	6	61	83
7	10.3	90	5	6	78	89
8	3.4	95	5	6	71	91
9	15.3	80	4	6	83	77
10’’	0.5	25	1	7	31	23
11’	2.5	80	3	6	52	85
Média/DP	6.86 ±6.04	72.78±22	3.67±1.22	6.22 ±0.44	59±16.67	70±22.36

’Animals 4 and 11 positive for *Brucella spp*. in semen; ”Animals 1, 4, 5, 6 e 10 positive for *Brucella spp.* in blood. ”Animal 4 was positive for *Brucella spp.* in blood and semen.

Table 2 -Testicular consistency, sperm concentration and sperm morphology (primary, secondary and total defects) of dogs from kennels in Cuiabá and Várzea Grande/MT, Brazil.

Animal	Consistency(1-3)	Concentration(x10^6/mL)	Primary Defects(%)	Secondary Defects(%)	Total Defects (%)
1”	3	20	4	20	24
2	3	-	-	-	-
4”’	3	13	2	35	37
5’’	3	38	1	31	32
6’’	2	20	2	25	27
7	3	42	6	16	22
8	3	54	2	13	15
9	2	48	5	14	19
10’’	3	19	4	29	33
11’	2	15	3	38	41
Média/DP	3±0.48	29.89 ±15.5	3.22±1.64	24.56±9.29	27.78±8.61

Animal 2 - not collected, ’Animals 4 e 11 positives for *Brucella spp.* in semen; ”Animals 1, 4, 5, 6 e 10 positives for *Brucella spp.* in blood.”Animal 4 was positive for *Brucella spp.* in blood and semen.

Nevertheless, it appears that the accuracy when using both materials is 58.33%, that is, it results in the same diagnosis in 14 animals and in different diagnoses in 10 (41.66%) animals. Additionally, the sensitivity was 40%, and the specificity was 71.43%.

When evaluating the PCR of blood and reproductive material by the Kappa value in table 4, we found that both reasonably agree on the result of the evaluation of *Brucella spp*. in the analyzed dataset. The McNemar test also showed that there is no difference between the error rates of the two tests to detect the presence or absence of the disease.

In males, paternity was confirmed in 8 (72.7%) animals, with 3 (33.3%) positive animals showing clinical signs. Male 3 (M3) had difficulty copulating due to a locomotor problem and was therefore castrated. Knowing that brucellosis can cause discoespondylitis, osteomyelitis and claudication (STUPAK et al., 2015; BUHMANN et al., 2019), we can presume that the reported problem originated from infection by *Brucella spp*. Male 7 (M7) had an eye infection, which is also a known clinical sign of brucellosis in dogs (LEDBETTER et al., 2009). Male 10 (M10) showed infertility, and sterility is one of the potential clinical signs of brucellosis (MINHARRO et al., 2005; HOLST et al., 2012; RODRIGUES et al. 2016; GARLACE et al., 2020).

The results of the PCR tests to detect *Brucella spp*. in males are shown in table 5. For the purpose of comparing the tests, only the results of 9 animals were used, which allowed the collection of material to perform the blood and semen tests. Two (18.2%) animals had a positive PCR result for *Brucella spp*. in semen, and 6 (54.5%) animals had a positive PCR result for *Brucella spp*. in blood, and of these, only 1 (9.1%) animal had a positive PCR result for *Brucella spp*. In both blood and semen samples. These findings are possibly related to the initial stage of infection found at the time of material collection.

In relation to the tests performed, the accuracy of using both materials is 44.44%, that is, both result in the same diagnosis in 4 animals and result in different diagnoses in 5 (55.56%) animals. Furthermore,

Table 3 - PCR result of *Brucella spp*. in blood and vaginal swab samples from females.

Blood	----------Swab----------		Total
	Positive	Negative	
Positive	4	4	8
Negative	6	10	16
Total	10	14	24

Table 4 - Kappa and McNemar tests with 95% confidence for the exams performed on females.

---------Agreement Kappa----------			--------McNemar---------	
P-value	Value	Agreement	P-value	χ^2
0.2928	0.1176	Weak	0.7518	0.1

Table 5 - PCR result of *Brucella spp*. in blood and semen samples from males.

Blood	----------Semen----------		Total
	Positive	Negative	
Positive	1	4	5
Negative	1	3	4
Total	2	7	9

the sensitivity was 50%, and the specificity was 42.86%.

In relation to the Kappa value, table 6 shows that the agreement between the tests is insignificant when evaluating the detection of *Brucella spp*. in the analyzed dataset; that is, the tests differ in terms of the results. The McNemar test also shows that there is no difference between the error rate of the two tests to detect the presence or absence of the disease.

The andrological examination was also negatively impacted by infection; animals that had positive semen and/or blood PCR results showed lower motility, vigor and concentration. Animals with positive semen and/or blood PCR results had lower values for membrane and acrosome integrity, indicating a negative influence of *Brucella spp*. on the sperm quality of these animals.

Following Blom's methodology (BLOM et al., 1972), the major defects found were as follows: small and abnormal sperm head and acrosome; proximal droplet and rudimentary intermediate piece, and strongly bent tail in the intermediate piece; and tightly curled tail in the main piece. Minor defects were distal drop and bent tail. Major and minor defects were proportionally higher in animals positive for *Brucella*.

Regardless of sex and material collected, when comparing the number of positive animals to those that showed clinical signs, we observed that most clinical signs affected the reproductive system, which makes clinical diagnosis difficult in animals that are not breeding or are castrated (KEID, 2018), which increases risk for the kennel as they continue to release the bacteria (CHACÓN-DÍAZ et al., 2015).

Table 6 - Kappa and McNemar tests with 95% confidence for tests performed on males.

----------Agreement Kappa----------			---------McNemar-------	
P-value	Value	Agreement	P-value	χ^2
0.2928	0.1176	Weak	0.3711	0.8

It was not possible to track the animals that were in the evaluated kennels when the study was performed; however, 15 (63.6%) males and 7 (62.5%) females came from other kennels, which may have led to the entry of infected animals into the sites evaluated. MOORE et al. (1970) noted that the spread of canine brucellosis is associated with the movement of dogs from one area to another. Another likely way of entry may be through the movement of breeding animals, especially males that are often used to cover females from other properties, thereby allowing disease transmission to these dams and subsequent dissemination of canine brucellosis in their kennel of origin.

CONCLUSION

Based on the results obtained, it was possible to diagnose *Brucella spp*. in males and females by PCR using the different biological samples analyzed, indicating that brucellosis is present in commercial kennels in the metropolitan region of Cuiabá.

According to the findings of the present study, vaginal swab PCR is an effective test to identify *Brucella spp*. in females; however, in males, blood PCR yielded more positives, possibly because these animals were at the beginning stage of infection.

ACKNOWLEDGEMENTS

The authors would like to thank the Laboratório de Pesquisa em Animais de Zoológico and the Laboratório de Microbiologia e Biologia Molecular da Universidade Federal do Mato Grosso (UFMT), Cuiabá – MT, Brasil, for their support with the PCR analyses and would like to thank Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES), Brasil - CAPES for the financial support.

DECLARATION OF CONFLICT OF INTEREST

The authors declare no conflict of interest. The funding sponsors had no role in the experimental design, data collection, analyses, or interpretation, manuscript writing, or in the decision to publish the results.

AUTHORS' CONTRIBUTIONS

All authors contributed equally to the conception and writing of the manuscript. All authors critically revised the manuscript and approved the final version.

BIOETHICS AND BIOSSECURITY COMMITTEE APPROVAL

This study was conducted with approval from the institutional Animal Use Ethics Committee (CEUA/UFMT; no. 23108.097448/2021-78).

REFERENCES

AGUIAR, D. M. et al. Anti-Brucella abortus and anti-Brucella canis antibodies occurrence in rural and urban dogs from Monte Negro county, Rondônia, Brazil. **Ciência Rural**, Santa Maria, v.35, n.5, p.1216-1219, 2005. Available from: <https://doi.org/10.1590/S0103-84782005000500039>. Accessed: Mar. 20, 2019. doi: 10.1590/S0103-84782005000500039.

ALVES, C. J. et al. Epidemiological aspects of Brucella canis in Patos, Paraíba, Brazil. **Ciência Rural**, Patos, v.13, n.1, p.45-49, 2003. Available from: <https://www.yumpu.com/pt/document/view/15581384/1-epidemiological-aspects-of-brucella-canis-in-patos-paraiba-brazil>. Accessed: Jul. 19, 2021.

ALMEIDA, A. C. et al. Seroepidemiology of canine brucellosis caused by Brucella canis and Brucella abortus in Alfenas, MG, Brazil. **Arquivo Brasileiro de Medicina Veterinária e Zootecnia**, Alfenas, v.56, n.2, p.275-276, 2004. Available from: <https://doi.org/10.1590/S0102-09352004000200021>. Accessed: Mar. 26, 2019. doi: 10.1590/S0102-09352004000200021.

BUHMANN, G. et al. Canine Brucellosis : Insights Into the Epidemiologic Situation in Europe. **Frontiers in Veterinary Science**, v.6, n.151, p.1–9, 2019. Available from: <https://doi.org/10.3389/fvets.2019.00151>. Accessed: Oct. 15, 2020. doi: 10.3389/fvets.2019.00151.

BLOM, E. et al. Congenital absence of the epididymis, ductus deferens and glândula vesicularis (aplasia segmentalis ductus wolfii) in the bull. **Copenhagen: Royal Veteninary and Agricultural University**, 1972.

CAVALCANTI, L. A. et al. Pesquisa de anticorpos anti-Brucella canis em cães provenientes da região metropolitana de Salvador**. Revista Brasileira de Saúde e Produção Animal**, Salvador, v.7, n.2, p.176-180, 2006. Available from: <https://repositorio.ufba.br/handle/ri/1914>. Accessed: Feb. 8, 2019.

CARDOSO P. G. et al. Brucella *spp*. noncanonical LPS: structure, biosynthesis, and interaction with host immune system**. Microbial Cell Factories**, London, v.5, n.13, 2006. Available from: <https://pubmed.ncbi.nlm.nih.gov/16556309/>. Accessed: Jan. 22, 2019. doi: 10.1186/1475-2859-5-13.

CARMICHAEL, L. E. Abortions in 200 Beagles. **Journal of American Veterinary Medical Association**, London, v.149, n.8, p.1126, 1966. Available from: <https://www.jstor.org/stable/30108493>. Accessed: Apr. 10, 2019.

CARMICHAEL, L. E. et al. Characteristics of a newly-recognized species of Brucella responsible for infectious canine abortions. **The Cornell Veterinary**, London, v.48, n.4, p.579-592, 1968. Available from: <https://pubmed.ncbi.nlm.nih.gov/5693645/>. Accessed: Apr. 19, 2021.

CHINYOKA, S. et al. Serological survey of Brucella canis in dogs in urban Harare and selected rural communities in Zimbabwe. **Journal of the South African Veterinary Association**. Namibia, v.85, p.1-5, 2014. Available from: <http://www.scielo.org.za/pdf/jsava/v85n1/09.pdf>. Accessed: Mar. 14, 2019. doi: 10.4102/jsava.v85i1.1087.

COLEGIO BRASILEIRO DE REPRODUÇÃO ANIMAL, **Manual para exame andrológico e avaliação de sêmen animal**. 3. ed. Belo Horizonte: CBRA, 2013.

CORBEL, M. J. Brucellosis: an overview. **Emerging Infectious Diseases**, Hertfordshire, v.3, n.2, p.213-221, 1997. Available from: <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2627605/>. Accessed: May, 20, 2021. doi: 10.3201/eid0302.970219.

CORRENTE, M. et al. Detection of Brucella canis in a dog in Italy. **The New Microbiologica,** Bary, v.33, n.4, p.337-341, 2010. Available from: <https://pubmed.ncbi.nlm.nih.gov/21213592/#:~:text=B.,canis%20in%20Italy>. Accessed: Jun. 10, 2020.

DORNELES, S. E. M. et al. Antibodies anti-Brucella canis and anti-Brucella abortus in dogs from Araguaína, Tocantins, Brazil. **Brazilian Journal of Veterinary Research and Animal Science**, Tocantins, v.48, n.2, p.167-171, 2011. Available from: <file:///C:/Users/Sara/Downloads/34369-Article%20Text-40321-1-10-20120722.pdf>. Accessed: Oct. 14, 2021.

DREER, M. K. P. et al. Toxoplasmosis, leptospirosis and Brucellosis in stray dogs housed at the shelter in Umuarama municipality, Paraná, Brazil. **Journal of Venomous Animals and Toxins including Tropical Diseases**, Paraná, v.19, n.1, 2013. Available from: <https://doi.org/10.1186/1678-9199-19-23>. Accessed: May, 19, 2020. doi: 10.1186/1678-9199-19-23.

FERNANDES, A. R. F. et al. Serological and molecular survey of canine brucellosis in the county of Natal, Rio Grande do Norte state. **Ciência Rural**, Patos, v.43, n.9, p.1629-1635, 2013. Available from: <https://doi.org/10.1590/S0103-84782013000900015>. Accessed: Jun. 13, 2019. doi: 10.1590/S0103-84782013000900015.

FERREIRA T. et al. Serodiagnosis of canine brucellosis using external and internal antigensof Brucella canis and Brucella ovis. **Revista Brasileira de Ciência Veterinária**, Niterói, v.14, n.3, p.167-168, 2007. Available from: <http://dx.doi.org/10.4322/rbcv.2014.256>. Accessed: Apr. 16, 2020. doi: 10.4322/rbcv.2014.256.

GALARCE, N. et al. Prevalence and Genomic Characterization of Brucella canis Strains Isolated from Kennels, Household, and Stray Dogs in Chile **Animals**, v.10, n.2073, p.1–13, 2020. Available from: <https://doi.org/10.3390/ani10112073>. Accessed: Oct. 06, 2021. doi: 10.3390/ani10112073.

GARDNER, E. et al. No evidence of Brucella canis infections in New Zealand dogs. **Surveillance**. New Zealand, v.24, p.17–18, 1997.

GOMES, A. S. **Programa Nacional de Sanidade Suídea (PNSS)**. Brasil: Ministério da Agricultura, Pecuária e Abastecimento. 2009. 441p.

GYURANECZ, M. et al. Detection of Brucella canis-induced reproductive diseases in a kennel. **Journal of Veterinary Diagnostic Investigacion**, Budapest, v.23, n.1, p.143-147, 2011. Available from: <https://journals.sagepub.com/doi/pdf/10.1177/104063871102300127>. Accessed: May, 22, 2021. doi: 10.1177/104063871102300127.

HENSEL, M. E. et al. Brucellosis in dogs and public health risk. **Emerging infectious diseases**, v.24, i.8, p.1401–1406, 2018. Available from: <https://pubmed.ncbi.nlm.nih.gov/30014831/>. Accessed: Jun. 18, 2020. doi: 10.3201/eid2408.171171.

HOLST, B. S. et al. The first case of Brucella canis in Sweden: background, case report and recommendations from a northern European perspective. **Acta Vet Scand**. v.54, n.1, p.1-9, 2012.

Available from: <https://pubmed.ncbi.nlm.nih.gov/22452858/>. Accessed: Apr. 28, 2019. doi: 10.1186/1751-0147-54-18.

HUBBARD, K. et al. Seroprevalence of brucellosis in Mississippi shelter dogs. **Prev Vet Med.**, Mississippi State, v.159, p82-86, 2018. Available from: <https://doi.org/10.1016/j.prevetmed.2018.09.002>. Accessed: Jan. 20, 2019. doi: 10.1016/j.prevetmed.2018.09.002.

JAMES, D. R. et al. Clinical management of Brucella suis infection in dogs and implications for public health. **Aust Vet J.**, v.95, p.19-25. 2017. Available from: <https://pubmed.ncbi.nlm.nih.gov/28124423/>. Accessed: Jan. 30, 2019. doi: 10.1111/avj.12550.

KEID, L. B. et al. Brucella canis infection in dogs from commercial breeding kennels in Brazil**. Transboundary and Emerging Disease**, Pirassununga, v.0, p.1–7, 2017. Available from: <https://pubmed.ncbi.nlm.nih.gov/28296215/>. Accessed: Sep. 17, 2019. doi: 10.1111/tbed.12632.

KEID, L. B. et al. Brucella *spp*. isolation from dogs from commercial breeding kennels in São Paulo state, Brazil. **Brazilian Journal of Microbiology,** Pirassununga, v.35, p.161-166, 2004. Available from: <https://doi.org/10.1590/S1517-83822004000100027>. Accessed: Nov. 10, 2019. doi: 10.1590/S1517-83822004000100027.

KIM, J. W. et al. Evaluation of immunochromatographic assay for serodiagnosis of Brucella canis. **The Journal of Veterinary Medical Science**, Anyang, v.69, n.11, p.1103-1107, 2007. Available from: <https://pubmed.ncbi.nlm.nih.gov/18057823/>. Accessed: Feb. 20, 2019. doi: 10.1292/jvms.69.1103.

LAGE, A. P. et al. **Programa nacional de controle e erradicação da brucelose e da tuberculose animal (PNCEBT): manual técnico**. Brasília: Ministério da Agricultura, Pecuária e Abastecimento (MAPA), 2006. 184 p.

LARSSON, M. H. M. A. et al. Brucelose canina experimental: Estudo Bacteriológico, Sorológico e Anatomopatológico. **Arquivo Brasileiro de Medicina Veterinária e Zootecnia**, Belo Horizonte, v.36, p.141–156, 1984.

LEDBETTER, E. C. et al. Brucella canis endophthalmitis in 3 dogs: clinical features, diagnosis, and treatment. **Veterinary Ophthalmology**, v.12, n.3, p.183-191, 2009. Available from: <https://pubmed.ncbi.nlm.nih.gov/19392878/>. Accessed: Feb. 14, 2019. doi: 10.1111/j.1463-5224.2009.00690.

LUCERO, N. E. et al. Brucella isolated in humans and animals in Latin America from 1968 to 2006. **Epidemiol Infect**. Buenos Aires, v.136, n.4, p.496-503, 2008. Available from: <https://pubmed.ncbi.nlm.nih.gov/17559694/>. Accessed: Feb. 8, 2019. doi: 10.1016/S0022-474X(00)00016-3.

MAPA. **Ministério da Agricultura Pecuária e Abastecimento**. Available from: <https://www.gov.br/agricultura/pt-br>. Accessed: Nov. 12, 2022.

MINHARRO, S. et al. Diagnóstico da brucelose canina: dificuldades e estratégias. **Revista Brasileira de Reprodução Animal**, v.29, n.3-4, p.167–173, 2005. Available from: <http://cbra.org.br/pages/publicacoes/rbra/download/pag%20167%20v29n3-4.pdf>. Accessed: Jan. 8, 2019.

MOL, J. P. S. et al. Diagnosis of canine brucellosis : comparison of various serologic tests and PCR. **Journal of Veterinary Diagnostic Investigation**, p.1–10, 2019. Available from: <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7003229/pdf/10.1177_1040638719891083.pdf>. Accessed: Nov. 30, 2021. doi: 10.1177_1040638719891083.

MONTEIRO, K. S. et al. Brucelose canina: uma revisão de literatura. **Scire Salutis, Lavras**, v.10, n.3, p.73-81, 2020. Available from: <https://doi.org/10.6008/CBPC2236-9600.2020.003.0009>. Accessed: Oct. 12, 2021. doi: 10.6008/CBPC2236-9600.2020.003.0009.

MOORE, J. A. et al. Epizootiology, diagnosis, and control of *Brucella canis*. **J Am Vet Med Assoc**, v.156, n.12, p.1737-1740, 1970. Available from: <https://pubmed.ncbi.nlm.nih.gov/4987213/>. Accessed: Oct. 13, 2019.

MOR, S. M. et al. Emergence of Brucella suis in dogs in New South Wales, Australia: clinical findings and implications for zoonotic transmission. **BMC Vet Res.** Sydney, v.12, n.1, p.1-9, 2016. Available from: <https://bmcvetres.biomedcentral.com/articles/10.1186/s12917-016-0835-0>. Accessed: Oct. 13, 2019. doi: 10.1186/s12917-016-0835-0.

OIE. **(World Organisation for Animal Health)**. Available from: <https://www.woah.org/en/home/>. Accessed: Nov. 27, 2022.

POPE, C. E. et al. A Simple Staining Method for Quantifying the Acrosomal Status of Cat Spermatozoa. **J. Zoo Wildl. Med**., v.22, n.1, p.87–95, 1991. Available from: <https://www.jstor.org/stable/20095123>. Accessed: May, 15, 2020. doi: 20095123.

R CORE TEAM. **A language and environment for statistical computing**. R Foundation for Statistical Computing, Vienna.

RODRIGUES, F. S. et al. Brucelose canina: revisão de literatura. **Revista Brasileira de Higiene e Sanidade Animal**, v.10, n.4, p.870-888, 2016. Available from: <http://www.higieneanimal.ufc.br/seer/index.php/higieneanimal/article/view/372>. Accessed: Jul. 22, 2019.

SANTOS, R. L. et al. Canine Brucellosis: An Update. **Frontier Veterinary Science. Belo Horizonte**, v.8, p.1-17, 2021. Available from: <http://C:/Users/Sara/Downloads/fvets-08-594291.pdf>. Accessed: Jun. 28, 2019. doi: 10.3389/fvets.2021.594291.

STUPAK, E. C. et al. Brucellosis Canine. **Revista Investigação**, São Paulo, v.14, n.6, p.113-117, 2015. Available from: <http://C:/Users/Sara/Downloads/1699-Texto%20do%20artigo-8972-1-10-20171127.pdf>. Accessed: Jan. 4, 2019. doi 8972-1-10-20171127.

SWANSON, E. W. et al. An eosin-nigrosin stain for differentiating live and dead bovine spermatozoa. **Journal of Animal Science**, v.10, p.981-987, 1951. Available from: <https://doi.org/10.2527/jas1951.104981x>. Accessed: Nov. 19, 2021. doi: 10.2527/jas1951.104981x.

UEDA, K. et al. K. Spontaneous Brucella canis infection in beagles: bacteriological and serological studies. Jpn**. J. Vet. Sci.,** v.36, n.5, p.381-389, 1974. Available from: <https://doi.org/10.1292/jvms1939.36.381>. Accessed: Dec. 3, 2019. doi: 10.1292/jvms1939.36.381.

VARGAS, A. C. et al. Canine brucellosis: a case report. **Ciência Rural**, v.26, n.2, 1996. Available from: <https://doi.org/10.1590/S0103-84781996000200024>. Accessed: Oct. 20, 2020. doi: 10.1590/S0103-84781996000200024.

VOLKWEIS, F. S. et al. Avaliação espermática e histológica dos testículos e epidídimos de caninos naturalmente infectados por B. canis. **Pubvet**, v.14, n.4, p.1–4, 2018. Available from: <https://doi.org/10.31533/pubvet.v14n4a543.1-4>. Accessed: Feb. 10, 2021. doi: 10.31533/pubvet.v14n4a543.1-4.

WANKE, M. M. Canine Brucellosis. **Animal Reproduction Science**. Buenos Aires, v.82, n.83, p.195-207, 2004. Available from: <https://pubmed.ncbi.nlm.nih.gov/15271453/>. Accessed: Sep. 6, 2021. doi: 10.1016/j.anireprosci.2004.05.005.

WHATMORE, A. M. et al. Second UK isolation of Brucella canis. **Vet Rec.**, Addlestone, v.180, n.25, p.617-618, 2017. Available from: <https://pubmed.ncbi.nlm.nih.gov/28646065/>. Accessed: Feb. 11, 2019. doi: 10.1136/vr.j3004.

ZHOU, K. et al. ONE Health Approach to Address Zoonotic Brucellosis: A Spatiotemporal Associations Study Between Animals and Humans. **Frontiers in Veterinary Science**, v.7, p.1–10, 2020. Available from: <https://www.frontiersin.org/articles/10.3389/fvets.2020.00521/full>. Accessed: Jul. 27, 2022. doi: 10.3389/fvets.2020.00521.