# Epidemiological indicators of canine piometra: An analysis of main components in

## the case of cases assisted in the northeast region of Pará

Indicadores epidemiológicos de piometra canina: Uma análise de componentes principais na

casuística de casos atendidos na região nordeste do Pará

Indicadores epidemiológicos de la piometra canina: Un análisis de los componentes principales en el caso de casos asistidos en la región noreste de Pará

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### Abstract

Pyometra is the endometrial disorder of greatest clinical importance in small animals. The realization of studies with the purpose of obtaining epidemiological data related to the occurrence of pyometra in bitches are fundamental. The objective was to determine epidemiological indicators of canine pyometra using the statistical method of principal component analysis and to characterize the set of parameters that facilitate the diagnosis and a possible clinicalepidemiological profile, through the casuistry attended in university veterinary hospitals in the northeastern region of Pará. A total of 142 medical records in two university veterinary hospitals were consulted. A total of 18 variables were analyzed using principal component analysis (PCA) and descriptive statistics. The PCA identified three groups of epidemiological indicators that together explained 71.05% of the variability of the data. The first group (abdominal pain, abdominal distension, critical condition and leukocytosis), the second (apathy, anorexia, pyometra type, and vaginal discharge) and the third (age, race, parity and pseudocyesis). It was observed that 47.7% of the bitches were five to 10 years old, 63.8% were of undefined breed and 62.4% were nulliparous (p < 0.05). The pyometra of the open type presented a frequency of 82.4% (p < 0.05). The variables pain, abdominal distension and leukocytosis are indicators of severe stage disease. The variables apathy, anorexia/hyporexia and vaginal secretion are indicators of disease in mild stage and the variables age and race act as epidemiological indicators of predisposition. Moreover, it was identified that the clinical-epidemiological profile of affected animals is of middle-aged, non-breed, nulliparous bitches with open pyometra.

Keywords: Canine; Castration, Epidemiology; Cystic endometrial hyperplasia.

### Resumo

A piometra é a desordem endometrial de maior importância clínica em pequenos animais. A realização de estudos com intuito de obtenção de dados epidemiológicos relacionados à ocorrência da piometra em cadelas, são fundamentais. O objetivo foi determinar indicadores epidemiológicos da piometra canina utilizando o método estatístico de análise de componentes principais e caracterizar o conjunto de parâmetros que facilitem o diagnóstico e um possível perfil clinico-epidemiológico, através da casuística atendida em hospitais veterinários universitários na região nordeste do Pará. Foram consultados 142 prontuários em dois hospitais veterinários universitários. No total foram analisadas 18

variáveis, utilizando análise de componentes principais (ACP) e estatística descritiva. A partir da ACP identificou-se três grupos de indicadores epidemiológicos que juntos explicaram 71.05% da variabilidade dos dados. O primeiro grupo (dor abdominal, distensão abdominal, estado crítico e leucocitose), o segundo (apatia, anorexia, tipo de piometra, e secreção vaginal) e o terceiro (idade, raça, paridade e pseudociese). Observou-se que 47.7% das cadelas tinham de cinco a 10 anos, 63.8% sem raça definida e 62.4% nulíparas (p < 0.05). A piometra do tipo aberta apresentou frequência de 82.4% (p < 0.05). As variáveis dor, distensão abdominal e leucocitose são indicadores da doença em estágio severo. As variáveis apatia, anorexia/hiporexia e secreção vaginal são indicadores da doença em estágio leve e as variáveis idade e raça atuam como indicadores epidemiológicos de predisposição. Além disso, identificou-se que o perfil clinico-epidemiológico de animais acometidos é de cadelas de meia idade, sem raça definida, nulíparas com piometra do tipo aberta.

Palavras-chave: Caninos; Castração; Epidemiologia; Hiperplasia endometrial cística.

#### Resumen

La piometra es el trastorno endometrial de mayor importancia clínica en los pequeños animales. La realización de estudios con el fin de obtener datos epidemiológicos relacionados con la ocurrencia de la piometra en perras, son fundamentales. El objetivo fue determinar indicadores epidemiológicos de la piometra canina mediante el método estadístico de análisis de componentes principales y caracterizar el conjunto de parámetros que facilitan el diagnóstico y un posible perfil clínico-epidemiológico, a través de la casuística atendida en los hospitales veterinarios universitarios de la región nordeste de Pará. Se consultaron 142 historias clínicas en dos hospitales veterinarios universitarios. Se analizaron un total de 18 variables mediante el análisis de componentes principales (ACP) y estadísticas descriptivas. A partir del ACP se identificaron tres grupos de indicadores epidemiológicos que, en conjunto, explicaban el 71.05% de la variabilidad de los datos. El primer grupo (dolor abdominal, distensión abdominal, estado crítico y leucocitosis), el segundo (apatía, anorexia, tipo de piometra y flujo vaginal) y el tercero (edad, raza, paridad y pseudociesis). Se observó que el 47.7% de las perras tenían entre 5 y 10 años, el 63.8% eran de raza no definida y el 62.4% eran nulíparas (p < 0.05). La piometra abierta mostró una frecuencia del 82.4% (p < 0.05). A partir de los resultados se identificó que las variables dolor y distensión abdominal y leucocitosis son indicadores de enfermedad en fase grave. Que las variables apatía, anorexia/hiporexia y flujo vaginal son indicadores de enfermedad en fase leve y que las variables edad y raza actúan como indicadores epidemiológicos de predisposición. Además, se pudo identificar que el perfil clínico-epidemiológico de los animales afectados es de perras nulíparas de mediana edad, no reproductoras, con piometra aberta.

Palabras clave: Caninos; Castración; Epidemiologia; Hiperplasia endometrial quística.

## **1. Introduction**

Brazil has a dog population estimated at 54 million animals, and approximately half of Brazilian families have at least one animal of this species in their homes (IBGE, 2013). Among the Brazilian states, the state of Pará holds about 1.8 million animals (Junqueira, 2017). Modern society has undergone constant changes, which includes the development of a closer relationship between humans and pets. In view of this, this circumstance makes this species an important component in social relationships (Khalid & Naqvi, 2016). Consequently, animal welfare characteristics, which include medical intervention, in order to increase the longevity of these animals have been the subject of scientific research (Stalliviere et al., 2013; Siqueira & Santis, 2020).

Reproductive system diseases play an important role among the pathologies that affect the canine species, and among these, uterine pathologies, especially pyometra, represent most of the cases diagnosed in female dogs, considered as a frequent disease in the pet clinic (Egenvall et al, 2001; Ortega-Pacheco et al, 2012; Maya-Pulgarin et al., 2017; Costa et al., 2019).

Canine pyometra also called cystic endometrial hyperplasia is a serious uterine infection, is characterized by a frequent and potentially fatal disorder (Egenvall et al, 2001; Gibson et al., 2013; Jitpean et al., 2016). The occurrence of cases of pyometra can be influenced by several epidemiological factors related to environmental conditions and reproductive aspects, factors such as age, race, parity, ovarian disorders and use of contraceptive methods can influence the occurrence of cases (Hagman et al., 2011; Ramos & Leite, 2016).

The realization of studies with the purpose of obtaining epidemiological data related to the occurrence of cases of pyometra in bitches, become fundamental in the establishment of new guidelines on prevention, diagnosis and treatment,

primarily due to the lack of such data in certain regions of the country. From this, the present study aimed to determine epidemiological indicators of canine pyometra, using the statistical method of principal component analysis, evaluating the casuistry seen at university veterinary hospitals in the northeastern region of the state of Pará. Thus, to trace the set of parameters that facilitate the diagnosis and a possible clinical-epidemiological profile.

## 2. Methodology

The epidemiological indicators were defined from the data survey of cases seen at two university veterinary hospitals located in the northeastern region of the state of Pará, being the Federal University of Pará (UFPA) and the Federal Rural University of the Amazon (UFRA) (Figure 1). To obtain the data, the records of the computerized systems of the veterinary hospitals involved were consulted in order to obtain information regarding the cases seen between January 2017 and December 2019. Given the non-use of animals and the use of existing data in the computerized systems of these places, the study was conducted under prior waiver of opinion issued by the Ethics Committee on the Use of Animals in Research (CEUA) of the institutions involved.

Figure 1 - Location of the veterinary hospitals where the data survey on incidence and prevalence of pyometra in female animals was conducted.





Data from 142 bitches with pyometra were analyzed using the following inclusion criteria: Signs of systemic disease (fever, anorexia, apathy, polyuria/polypsy, emesis and abdominal distension), presence of vaginal discharge of purulent/mucopurulent aspect during physical evaluation for cases of open pyometra and/or upon macroscopic evaluation of intrauterine fluid for cases of closed pyometra, and in abdominal ultrasound examination the presence of cystic and hyperplastic endometrium with increased uterine diameter and presence of luminal content. A total of 18 variables were analyzed (Board 1), after analysis they were tabulated in Excel spreadsheet and analyzed by multivariate statistics and descriptive analysis.

**Board 1** - Variables analyzed from the computerized systems of the veterinary hospitals of the Federal University of Pará and Federal Rural University of the Amazon during the period 2017-2019.

Variables analyzed from the computerized system (UFRA-UFPA)					
1.	Age	10. Leukocytosis			
2.	Race	11. Type of treatment			
3.	Parity	12. Abdominal pain			
4.	Contraceptive use	13. Abdominal distension			
5.	Pseudocieses	14. Apathy			
6.	Critical condition	15. Anorexia/hyporexia			
7.	Type of pyometra	16. Emesis			
8.	Elevation in ALP and AAT levels	17. Polyuria/Polypsy			
9.	Elevation in Urea and creatinine levels	18. Vaginal discharge			

Source: Authors.

For multivariate analysis it was employed the statistical method of factor analysis/main component analysis (FA/PCA). The PCA was chosen as a statistical method because it is considered a suitable tool for simultaneous studies of two or more variables (Inei, 2002), providing a better interpretation of the totality of the data.

From the 18 variables evaluated, it was possible to extract 12 that proved to be significant in the definition of the principal component analysis (PCA) model. The extraction of the correlation matrix enabled the identification of the association between variables, which could show the general trend of the data, as well as the variables of greater significance for the study proposal. The next step consisted of the decomposition of the correlation matrix to reduce the dimension of interrelated variables in smaller dimension, formed by common and independent factors (principal component). The model that best fitted the data was that composed of three components with characteristic roots greater than unity. The KMO (Kaiser-Meyer-Olkin) sampling adequacy measure test and Bartlett's test of sphericity were applied to adjust the parameters to the PCA (p<0.05). The selection of the number of components to be extracted follows the precepts presented by Norusis, (1990) that considers only components with eigenvalue greater than one. The multivariate analysis was performed by the program SPSS®, v.25.

Univariate statistics was applied to data categorized as non-parametric, using the chi-square test (p < 0.05), where the null hypothesis considered that the differences within each category would not be significant. When comparison by chi-square was necessary in more than two responses, the test was applied in each pair to assess differences. For the analysis it was used the free package of the program SPSS® v.25 (Statistical Package for the Social Sciences).

For descriptive analysis the variable age was categorized into three categories, young bitches up to five years old, middle age bitches from five to 10 years old, and older bitches over 10 years old. To analyze the variable contraceptive use was considered the information obtained at the time of anamnesis, was given as presence for bitches who had already made use of hormonal contraceptives at some stage of life and absence for those who had never used. The variable parity was categorized into three categories, nulliparous, primiparous and multiparous. To analyze the variable pseudocyesis, the previous history of the patient was taken into consideration, considered as the presence of pseudocyesis for female dogs in which the guardians reported during the anamnesis that the animal had already presented clinical signs of pseudocyesis in some phase of its life. The animals that presented more severe signs of the disease such as dehydration, hypothermia, toxemia

and sepsis at the time of diagnosis were considered as critically patients. The variables referring to the alterations found in the hematological exams as presence of leukocytosis and elevation in the levels of urea, creatinine, alkaline phosphatase (ALP) and alanine aminotransferase (AAT) were obtained from the analysis of the hemogram and serum biochemistry exams of the patients. The cases that presented elevation in urea and creatinine levels were considered as presence of azotemia and the cases with elevation in ALP and AAT levels were considered as presence of vaginal discharge was observed classified as open pyometra, and for cases with absence of vaginal discharge classified as closed pyometra. When performing the tabulation of data, it was observed that in some cases there were missing data, in these cases the data was tabulated as unidentified variable.

## 3. Results

From the results obtained by the principal components analysis it was possible to determine three groups of epidemiological indicators, represented by three principal components that together were able to explain 71.05% of the variability of the data (Table 1). The first group of indicators represented by CP1 (Indicators of pyometra severity) explained 29.2% of the total variance of the data, within this group are contained four variables, being the heaviest, presence of pain and abdominal distension, followed by patient in critical condition and signs of leukocytosis. The second group of epidemiological indicators represented by CP2 (Indicators of pyometra in mild stage) was responsible for explaining 24.05% of the total variability of the data, in this group are contained the variables apathy, anorexia/hyporexia, vaginal discharge and type of pyometra. The third group of indicators in turn, represented by CP3 (Predisposition indicators), was responsible for explaining 17.75%, in this last group are contained variables such as age, race, parity and pseudocyesis.

Variables	CP1	CP2	CP3
Age	0.285	0.158	0.758
Race	0.159	0.166	0.731
Parity	0.127	0.184	0.556
Pseudocieses	0.511	0.208	0.563
Apathy	-0.214	-0.975	0.104
Anorexia/hyporexia	0.066	0.876	0.179
Abdominal distension	0.832	0.230	0.216
Abdominal pain	-0.870	0.046	0.155
Critical condition	0.659	0.107	-0.076
Leukocytosis	0.495	-0.084	0.106
Type of pyometra	0.149	-0.897	0.067
Vaginal discharge	0.018	0.975	-0.038
explained variance (%)	29.25	24.05	17.75
cumulative variance (%)	29.25	53.3	71.05

**Table 1 -** Epidemiological indicators groups of pyometra from principal component analysis of cases seen at UFPA and UFRAveterinary hospitals during the period 2017-2019.

Source: Authors.

From the results obtained by descriptive analysis it was observed a higher occurrence (p < 0.05) in middle age bitches

(5-10 years), followed by young bitches (up to 5 years) and lastly, elderly bitches (above 10 years) (Table 2). The prevalence of pyometra was higher in mixed breed (MBD) bitches (63.8%), followed by poodle (9.9%), York Shire and pinscher (6.3%) Dachshund, Rottweiler and Pit bull (2.1%), Golden and Shih tzu (1.4%), and other breeds (4.6%) (data not shown).

**Table 2** - Occurrence rates (%) of different age groups in female dogs diagnosed with pyometra seen at UFPA and UFRA veterinary hospitals during the period 2017-2019.

-	Group (age)	No. of cases Pyometra	Occurrence (%) *
-	Young (up to 5 years)	39	30.0b
	Middle age (5-10 years)	62	47.7a
*	Elderly (above 10 years)	29	22.3b

Chi-square test performed by pairwise testing. Source: Authors.

Regarding parity, a higher incidence (p < 0.05) was observed in nulliparous bitches when compared to the groups of primiparous and multiparous bitches (Table 3). As for the variable pseudocyesis, a low prevalence of 7.7% of patients with a history was identified (p < 0.05). The use of exogenous hormonal contraceptives was observed in 46.8% of the bitches, with at least one contraceptive administration. Among the group of animals that had already used hormonal contraceptives, there was a prevalence of 51.8% of young animals (up to 5 years), followed by middle age animals (5 to 10 years) (31.4%) and Elderly animals (above 10 years) (16.6%). As for the variables related to the alterations in the hematological examinations, a prevalence of 66.7% (p < 0.05) of leukocytosis cases was identified, low prevalence in the elevation of ALP and AAT levels (12.8%), indicating hepatopathy in few animals and 21.3% of elevation in urea and creatinine levels indicating azotemia in few animals.

 Table 3 - Incidence rates (%) of nulliparous, primiparous and multiparous bitches with diagnosis of pyometra seen at UFPA and UFRA veterinary hospitals during the period 2017-2019.

Group (Parity)	No. of cases Pyometra	Incidence (%) *
Nulliparous	78	62.4a
Primiparous	18	14.4b
Multiparous	29	23.2b

\* Chi-square test performed by pairwise testing. Source: Authors.

In relation to the classification of pyometra type, it was identified a prevalence of 82.4% (p < 0.05) of cases of open pyometra and only 17.6% of cases of the closed type. About 72% (p < 0.05) of patients with closed pyometra were in critical condition at the time of initial clinical care. The main clinical signs identified were apathy (94.4%), anorexia/hyporexia (90.1%), vaginal discharge (83%), abdominal discharge (67.9%), abdominal pain (58.6%), emesis (33.8%) and polyuria/polypsy (15.5%). Regarding the type of treatment used, it was observed that in 100% of the cases surgical treatment was used with the ovariohysterectomy technique.

### 4. Discussion

Based on the results obtained by the principal components analysis it was possible to determine that the three components together were able to explain 71.05% of the variability of the data, showing that the variables contained in these components are closely related to the occurrence of cases. The choice of the three components was defined by the principle that at least 70% of the total variance of the data should be explained (Rencher, 2002). From this, it can be observed that the PCA was used to reduce the dimensions of the original variables without loss of information and thus define the epidemiological indicators of canine pyometra in the northeastern region of Pará from the variables contained in these components.

The first epidemiological indicators were defined by CP1, within this component four variables are contained, with the highest weight, presence of pain and abdominal discharge, followed by the patient in critical condition and signs of leukocytosis, showing that the presence of these clinical and hematological signs correlate with the most severe cases associated with pyometra of the closed type. Since this is considered the most severe form of the disease because the uterine secretion is not drained, causing an increase in uterine diameter and consequent endotoxemia, increasing the risk of systemic inflammatory response syndrome (Jitpean et al., 2016), justifying the presence of signs such as abdominal distension due to uterine enlargement and leukocytosis.

From the analysis of CP2 it was possible to determine that the variables apathy, anorexia and vaginal discharge are closely related to cases of open pyometra. Evidencing that these clinical signs can act as epidemiological indicators for cases of pyometra in bitches, especially for cases of open pyometra. This fact reaffirms the importance of identifying these clinical signs quickly and accurately in order to enable the establishment of an early diagnosis, considering that the most favorable prognosis in cases of pyometra is based on early diagnosis of the disease (Hagman, 2017; Costa et al., 2020).

In turn, PC3 explained 17.75% of the total variance of the data, thus it was possible to identify the epidemiological indicators related to predisposing factors for the occurrence of cases. It is important to note that within this component the variables age and race showed greater prominence, as described by authors who report that one of the most relevant risk factors for the development of pyometra is advanced age (Martins et al., 2002; Dyba et al., 2018; Hagman, 2018; Kumar et al., 2019), as well as, genetic characteristics of certain races (Kenney et al., 1987; Egenvall et al., 2001; Hagman et al., 2011; Jitpean et al., 2012).

The age range between 5 and 10 years was the one that presented the highest (p < 0.05) occurrence of pyometra in the animals of the study. This observation suggests that the prevalence of pyometra in animals in this age group increases as a result of changes due to chronic and repetitive exposure of the uterus to progesterone during the estrous phase (Gibson et al., 2013; Hagman, 2018; Kumar & Saxena, 2018; Solano et al., 2019). Moreover, it was identified that the second group with the highest occurrence was animals under five years of age (young animals), showing that pyometra can also affect young animals, and that it is probably associated with an abnormal response of the uterus to a prolonged phase of exposure to progesterone instead of repetitive exposures, as it occurs in older bitches (Hagman, 2004).

Although it has been observed a higher prevalence of MDB animals (63.8%), it is not possible to affirm that there is a predisposition as to the race, a fact that may be related to a higher frequency of animals mixed breed in the treatment routine of veterinary hospitals, especially the university hospitals, as in this study. The same was observed by Evangelista (2009) who identified a higher prevalence of MDB animals with pyometra (60%), justifying a higher occurrence of these in the hospital routine. However, the risk of developing pyometra is believed to be increased in large races such as; Rottweiler, Saint Bernard, Chow Chow, Golden Retriever, Labrador and German Shepherd in comparison with all other races, including dogs of mixed breeds (Smith, 2006; Hagman et al., 2011; Jitpean et al., 2014; Kumar et al., 2019).

Despite the identification of a higher prevalence of pyometra cases in nulliparous bitches, it cannot be said that the absence of previous pregnancy can act as a predisposing factor for the occurrence of pyometra cases. Being that, the high occurrence of nulliparity in the bitches of that study may be associated with the presence of endocrine disorders as high exposures of progesterone and estrogen, as well as, with the presence of bacterial infections by ascending route that occurs during the estrus phase (Hagman, 2012). In addition, a low prevalence of pseudocyesis cases (7.7%) was identified in the animals of this study, evidencing that the history of pseudogestation is not related to the onset of pyometra cases in bitches, as well as observed in a more recent study (Hagman et al., 2011).

The prevalence of use of hormonal contraceptive methods (46.8%,) showed no significant statistical value (p>0,05), it is believed that this fact is related to the increase in awareness of owners by veterinarians during clinical care in which they are informed about the adverse effects of the use of contraceptive methods (Santos & Rosa, 2016; Rédua & Bueno, 2020). When the age range was evaluated within the group of animals that had used contraceptives, a higher prevalence was observed in young animals (51.8%) (up to 5 years), showing that the use of exogenous hormonal contraceptive method may have acted as a pathogenic factor for the occurrence of cases in young animals. Contraceptive methods cause an increase in the number of progesterone receptors, stimulating leukocyte activity favoring bacterial contamination and thus the development of cystic endometrial hyperplasia (Smith, 2006; Oliveira & Marquez 2006; Ramos & Leite, 2016; Dyba et al., 2018).

Elevated total leukocyte count is one of the most important hematological alterations in patients diagnosed with pyometra (Santana et al., 2014), it is believed that this elevation occurs in response to the inflammatory/infectious condition, associated with a set of chemical mediators (Trall, 2007). However, although a high prevalence (66.5%) of leukocytosis cases was found in the patients of the study, this laboratory alteration was not considered as a pathognomonic finding, since the study showed patients with leukocyte counts within normality.

It was identified a prevalence of patients with elevation in ALP and AAT levels (12.8%) and urea and creatinine levels (21.3%), these elevations show that the animals may present different degrees of renal and hepatic impairment, occurring due to deposition of immunocomplexes in glomerulus (Kumar & Saxena, 2018). A drop in perfusion occurs due to hypovolemia and systemic hypotension, as well as, by lesion in hepatocytes caused by endotoxemia and/or reduced circulation in the liver from dehydration (Feldman & Nelson, 1996; Nunes et al., 2010; Griffiths & Kanagasundaram, 2011; Honório et al., 2017).

The pyometra of the open type is the most common and the less aggressive form of the disease, thus it presented a higher prevalence (p < 0,05) in the cases of the study, in which, it can be justified due to the easiness of perception of the guardian of the animal, what takes the same to seek veterinary help and, consequently, it increases the numbers of diagnosed cases in the veterinary clinic (Evangelista ,2009; Dyba et al., 2018). Although the closed pyometra type has presented a lower number of cases, the prevalence of patients in critical condition was higher in this group (p < 0.05), showing that the closed pyometra type is the most severe form, for being generally associated with the presence of more severe clinical signs associated with septicemia, toxemia or shock (Jitpean et al, 2016; Verstegen et al., 2008; Hagman & Greko, 2005).

Among the clinical signs identified, three presented higher prevalence (p < 0.05), being apathy (94.4%), anorexia/hyporexia (90.1%) and vaginal discharge (83%), evidencing these as the most common symptoms in patients diagnosed with pyometra, especially for being characteristic signs of open pyometra (Hagman et al., 2011; Jitpen et al., 2016; Dyba et al., 2018; Oliveira et al., 2019; Solano et al., 2019).

For the type of treatment used, it was observed that in all cases the ovariohysterectomy technique was recommended. This fact may be related to the number of patients in severe condition and the lack of interest in maintaining reproductive activity, moreover, this surgical technique is considered the treatment of choice for cases of pyometra (Fieni et al., 2014; McCobb et al.,

2022).

## **5.** Conclusion

Through principal component analysis it was possible to identify that the presence of clinical signs such as pain and abdominal distension associated with leukocytosis act as indicators of disease in severe stage, the presence of apathy, anorexia/hyporexia and vaginal discharge indicate the presence of disease in milder and earlier stages. The variables age and race act as predisposition indicators for the occurrence of cases of pyometra in bitches. In addition, it was possible to identify that the clinical-epidemiological profile of animals commonly affected in the Northeast region of Pará are adult bitches, middle-aged (5 to 10 years old), mixed breed and nulliparous, and that the open type pyometra is the most frequent. The presence of leukocytosis, apathy, anorexia/hyporexia and vaginal discharge are common findings in patients diagnosed with this condition and the use of exogenous hormonal contraceptives can act as a pathogenic factor for the occurrence of cases in young animals.

In future studies, the amount of data to be collected from hospital systems should be increased, as well as to include epidemiological factors not addressed in this study.

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