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RECOMBINANT CHIMERIC PROTEINS OF BARTONELLA HENSELAE AS A FELINE BARTONELOSE IMMUNODIAGNOSTIC

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ABSTRACT

Bartonellosis is a disease of great importance for unique health, but it presents a challenging diagnosis, since conventional techniques are limited, in relation to the high complexity and time required. Therefore, the search for new targets for the serological diagnosis of Bartonella henselae is of considerable interest and the development of specific antigens can increase the sensitivity and specificity of this diagnosis. Thus, this research evaluated the antigenic activity of recombinant chimeric proteins synthesized from immunogenic epitopes of B. henselae for immunodiagnosis of feline bartonellosis. To this end, the recognition activity of chimeras in 145 samples of feline sera was analyzed through indirect ELISA (ELISA feline Bartonella-EFB). To analyze the general characteristics of the test, an adherence analysis was performed, using the metrics of accuracy, sensitivity, specificity, and area under the ROC curve (AUROC). The sensitivity, specificity, positive predictive value, negative predictive value, and accuracy of the EFB for detection of anti-B antibodies. henselae were also measured using the indirect immunofluorescence (IFA) test to analyze its possible application as a screening and/or confirmation method in the serological diagnosis of feline bartonellosis. Chimeric proteins were effective in detecting the presence of antibodies against B. henselae in feline serum samples, and also, all bartonellosis reactive samples in the proposed test were also reactive in the gold standard test (IFA). As the proposed test demonstrated low sensitivity and high specificity, recombinant proteins showed promise, and can be used as a tool for the diagnosis of feline bartonellosis.

Keywords: Diagnosis. Cat-Scratch Disease. Ectoparasites. Chimeras. Zoonosis.

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INTRODUCTION

Vector-borne diseases are of paramount importance for veterinary and human medicine, since the interaction between species favors the transmission and dissemination of zoonoses [1]. The proper diagnosis of these diseases contributes to the timely treatment of animals, reducing the transmission of the agent to other animals and to humans. In this sense, silent zoonoses, such as bartonellosis, constitute a challenge, because the animals are often asymptomatic or oligosymptomatic, which hinders the early identification and treatment of sick animals, increasing the risk of transmission to other individuals [2].

Bartonellosis is caused by pathogenic bacteria of the genus *Bartonella*, a subclass α2 of proteobacteria, consisting of intracellular, aerobic, and slow-growing bacilli. Of the 46 known species of *Bartonella*, 18 are associated with human disease [3]. Regarding the species, in addition to *B. henselae,* the etiological agent of cat-scratch disease, which affects people and can cause severe and even fatal infection in immunocompromised individuals [4], *B. clarridgeiae and B. koehlerae*, are also associated with cats, considered important reservoirs and transmitters [5].

Transmission occurs mainly in vector form, by hematophagous insects, such as sandflies and fleas, with mammals being their natural reservoirs [6]. Fleas of the species *Ctenocephalides felis* are natural vectors of *Bartonella* spp. and transmission to felines occurs through the inoculation of fecal particles of fleas infected with the bacterium in pre-existing lesions or during *grooming*, enabling transmission to other animals or people through scratching or biting [1, 5, 6].

The diagnosis of bartonellosis is a challenge, as there is still no technique that detects the presence of infection with [2] accuracy. The most commonly used techniques are ELISA, *western blot*, indirect immunofluorescence, molecular analysis such as polymerase chain reaction (PCR), immunohistochemistry, isolation, and microbiological culture, however, all have limitations [2, 7]. Culture is considered the gold standard for confirmation of *Bartonella* infection, and specialized techniques such as enriched growth media and cell culture [8] are recommended. However, this technique requires a specialized laboratory, specific culture media, controlled atmosphere, and the slow growth of the microorganism requires more time for the results [9].

In view of the above, understanding and improving the methods of diagnosing bartonellosis is of great interest to the scientific community and to society, considering that infection in cats can be asymptomatic, or with nonspecific clinical signs and persistent

Revista ARACÊ
ISSN: 2358-2472

bacteremia, a fact that facilitates zoonotic transmission [10, 11]. Thus, the availability of new faster, more effective and low-cost diagnostic instruments is fundamental not only for human health but also for veterinary medicine, especially in felines from vulnerable communities with inadequate vector control. Therefore, this research evaluated a new method of serological diagnosis for bartonellosis in felines, with the use of recombinant chimeric proteins produced from B*. henselae antigens*, in addition to describing hematological and biochemical parameters associated with felines in the study.

MATERIAL AND METHODS

The study was developed at the Ceval Veterinary Outpatient Clinic, which is a clinical care center of the Veterinary Clinics Hospital (HCV) of the Federal University of Pelotas (UFPel), located in a region of social vulnerability in the city of Pelotas-RS, in the South Region of Brazil. In these spaces, during consultations, blood is usually collected from the animals to carry out tests in order to determine their health status, and the remaining samples are identified and stored in the serum library of the Clinical Pathology Laboratory of HCV/UFPel and made available for research. For this study, based on the information that practically 100% of the felines treated at HCV have ectoparasites at some stage of life, 145 feline serum samples were used, from 125 patients, The blood samples, in a volume of approximately 3mL, were collected aseptically, by jugular or cephalic puncture, identified and packed in sterile tubes without anticoagulant. Subsequently, the samples were centrifuged at 3,000 × g for 15 minutes. The serum samples obtained were transferred to 2 ml tubes and stored at -20ºC, until processing and use in laboratory analyses.

In addition, data related to the hematological and biochemical analyses of the animals, obtained through the HCV-UFPel management software (SimplesVet), which contains the complete history of all patients, were collected and tabulated to identify if they presented any alteration indicative of infection in the tests performed.

RECOMBINANT PROTEINS

In this study, three recombinant chimeric proteins called rQ1, rQ2 and rQ3 were analyzed, which were developed from epitopes identified based on the sequences GroEL, 17 kDa, P26, BadA, Pap31, OMP89 and OMP43, which are important antigens of *B. henselae*, as described by Gonçalves et al. [12]. According to the authors, these proteins

Revista ARACÊ
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were expressed and purified using a heterologous system based on *Escherichia coli* and reacted with antibodies present in the serum of naturally infected humans, which were provided for this research by the Laboratory of Bacteriology and Bioassays (LABBio/UFPel).

2.2 ANTIGENICITY OF RECOMBINANT CHIMERAS WITH FELINE SERUM

Western blot (WB) *assays were performed* to evaluate the recognition of rQ1, rQ2 and rQ3 proteins by anti-Bartonella antibodies that could be present in the serum of naturally infected felines. For this, the proteins were separated by polyacrylamide gel electrophoresis (SDS-PAGE 12%), electro transferred to nitrocellulose membranes and blocked with Fetal Bovine Serum (SFB 1%), for 1 h, under agitation (50 rpm). Afterwards, washing was performed with phosphate buffered *saline (Phosphate Buffered Saline* PBS-T) to remove the blocking solution.

Next, a pool of 28 feline sera not yet tested for bartonellosis (primary antibody) was analyzed, which was shaken for 1 hour at 50 rpm. Subsequently, washes were performed with PBS-T buffer to remove the primary antibody (feline serum). The anti-feline IgG conjugate antibody (Rhea Biotech) (secondary antibody) was added to the membrane at the concentrations defined by the manufacturer (1:10,000), with the objective of amplifying the detection of antibodies of interest, conferring greater specificity to the test. For development, a substrate/chromogen solution was used (6mg of diaminobenzidine, 0.03% of nickel sulfate, 50 mM of Tris HCl pH 8.0 and 0.03% of hydrogen peroxide).

ELISA FELINE BARTONELLA (EFB)

The indirect ELISA was developed using chimeric proteins and feline *anti-Bartonella* serum , here called ELISA Feline *Bartonella* (EFB), in polystyrene plates with 96 cavities sensitized with the recombinant chimeras individually, diluted in carbonate-bicarbonate buffer (50 mM; pH 9.6). The concentration of each antigen (5 µg/ml) as well as the dilution of the feline sera (1:100) were predetermined using the checkerboard ELISA [13]. After incubation for 1h at 37°C, the cavities were washed with saline phosphate buffer (PBS 1X) and blocked with 0.5% casein solution.

The feline sera were diluted in 1X PBS and incubated at 37°C for one hour in contact with the antigens. Four negative controls were used: i) only chimeric proteins; (ii) chimeric proteins and diluent; (iii) chimeric proteins and conjugate and (iv) conjugate only.

Revista ARACÊ
ISSN: 2358-2472

The anti-feline anti-IgG antibody conjugated with peroxidase (Rhea Biotech) was added in dilutions pre-established by the manufacturer (1:10,000). The polystyrene plates were kept at 37°C for 1 hour between each of the test steps. Washes were performed between each reaction, using PBS-T solution (PBS + 0.05% Tween 20). To visualize the reactions, 0.1 M citrate buffer was added plus 0.2 mg/mL of *O-Phenylenediamine Dihydrochloride* (OPD) and 0.03% of H2O2. Subsequently, a 2M H2SO4 solution was used to interrupt the reaction, which was quantified via a spectrophotometer with a wavelength of 492 nm.

All tests were performed in duplicate and all samples tested for each chimera (rQ1, rQ2 and rQ3). The samples identified as reagents were tested again in duplicate, 20 days after the reactive result.

COUNTER-TEST - SEROLOGICAL ANALYSIS (IFA-FIOCRUZ)

Serological analysis was used as a counter-proof of positive results in the proposed test (EFB) at the Hantavirus and Rickettsiosis Laboratory of the Oswaldo Cruz Institute (LHR), a regional reference service for the diagnosis of rickettsiosis, using indirect immunofluorescence (FAI) for the diagnosis of *B. henselae*, with the *Bartonella* diagnostic kit IgG API REF IF1300G, X13082N lot (FOCUS), following the laboratory protocol and the manufacturer's recommendations, with a cut-off point for reagent samples of 1:64. In the counter-test, eight feline serum samples selected from the samples analyzed for EFB randomly and the four reactive samples in the EFB without identification were analyzed, totaling twelve samples. The samples were diluted in 1:64 and 1:128 in phosphate salt buffer, the intensity of specific fluorescence was subjectively evaluated (with scores from 1 to 4) and the antibody titer was defined by the main dilution with score 2.

STATISTICAL ANALYSIS

EFB adherence was analyzed according to the metrics of accuracy (total number of correct answers/total number of records), sensitivity (total number of positive correct answers/total number of positive answers), specificity (total number of correct answers/total number of negative answers), and area under the ROC curve (AUROC) (sensitivity as a function of the proportion of false positives: 1 - specificity). To evaluate the differences between hematological and biochemical parameters between the animals, each result was compared using Generalized Linear Models (GLM's) according to the categories: positive

Revista ARACÊ
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in both tests; positive only at FIOCRUZ and negative in both tests. All tests were performed in the R [14], considering a significance index of 95%.

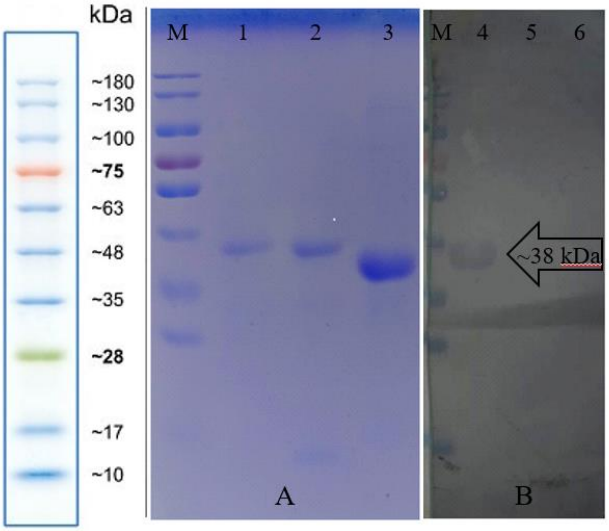
The sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV) and accuracy of the EFB method for detecting anti-B. *henselae* antibodies in feline serum samples were measured against the IFA, to analyze its possible use as a screening and/or confirmation method in the serological diagnosis of feline bartonellosis.

For the calculation of sensitivity, specificity, PPV, NPV and accuracy, reactive true samples (RV), non-reactive true samples (VNR), reactive false samples (RF), non-reactive false samples (FNR) of the ELISA method in relation to the IFA method were considered; reactive samples (VR + FR) and non-reactive samples (VNR + FNR) in relation to the ELISA method; reactive samples (R) and non-reactive samples (NR) in relation to the API method; and total samples analyzed (T).

RESULTS

Of the three purified chimeric proteins evaluated by *Western blot* (rQ1, rQ2 and rQ3) (Figure 1A), only rQ1 was recognized by serum from naturally infected felines (Figure 1B.

Figure 1. Expression and antigenicity of recombinant chimeric proteins rQ1, rQ2 and rQ3. *Note.:* (A) SDS-PAGE 12% stained with Comassie Blue and (B) *Western blotting* recognition of recombinant chimeras by anti-antibody*BartonellaElaela* Present in the serum of naturally infected felines. M - Molecular Weight Marker Page Ruler (Thermo Scientific), 1 4 - Q1; 2e 5 - Q2; 3e 6 - Q3, identified in the 38 kDa range.



Of the 145 feline serum samples analyzed by EFB, four were reactive against the chimeras evaluated (Table 1), which indicates that these felines were possibly infected by the bacterium, a result that was confirmed after the repetition of the test under the same

Revista ARACÊ
ISSN: 2358-2472

conditions. Thus, the recombinant chimeras developed through the prediction of epitopes by immunoinformatics tools reacted with antibodies present in feline serum, offering an interesting alternative for immunodiagnosis of bartonellosis.

Table 1. Results of feline sera submitted to the ELISA test *Feline Bartonella* (EFB).

	Feline Serum Sample 1	Feline Serum Sample 2	Feline Serum Sample 3	Feline serum sample 4
Protein Q1	Reagent	Non-reagent	Reagent	Non-reagent
Protein Q2	Reagent	Reagent	Reagent	Non-reagent
Protein Q3	Reagent	Non-reagent	Reagent	Reagent

Of the 12 samples sent for confirmation by IFA, eight were positive, and all samples reactive for *Bartonella* in the EFB (n = 4) also reacted in the IFA, but four non-reactive samples in the EFB reacted in the counter-test (Table 1). Comparing the results obtained in the EFB with the counter-test (IFA) we observed 4 true reactive samples, 4 false reactive negatives, 4 true non-reactive and none false reactive. These data are used to determine the sensitivity, specificity, PPV, NPV and accuracy of the EFB for the detection of *anti-Bartonella henselae* antibodies in feline serum samples measured using the IFA method (Table 2).

Table 2. Results of feline sera submitted to the IFA countertest for validation of the results obtained in the ELISA Feline *Bartonella* (EFB) test.

Asytra soros felines	Feline Bartonella *ELISA Test* (EFB)	IFA Contratest
1	+	+
2	-	+
3	+	+
4	-	-
5	-	-
6	-	+
7	+	+
8	+	+
9	-	-
10	-	-
11	-	+
12	-	+

*IFA: Indirect Immunofluorescence.

The sensitivity of the preliminary tests of the EFB in relation to the IFA was 50% and the specificity was 100%. The PPV, the number of positives in both tests over the number of positives in the EFB alone, was 100% and the NPV, calculated by the number of negative samples in both tests over the total number of negatives in the EFB test, was 50%. The accuracy, determined by the sum of the positive and negative sera detected in the two tests over the total sample, was 66.7%. The area under the ROC curve

Revista ARACÊ
ISSN: 2358-2472

demonstrates that despite the high specificity, i.e., the ability of the EFB to measure felines that are truly negative for bartonellosis, the sensitivity was low, showing that the test presents imprecision in the identification of those that are truly positive (EUROC=0.75), despite not presenting false negatives.

The blood samples of all the felines evaluated were also submitted to hematological and biochemical analyses, the results of which were compared between the animals that were not reactive in the two tests, reactive in the API and reactive in both tests, EFB and IFA (Table 3).

Table 3. Mean and standard deviation of the hematological and biochemical parameters of the felines analyzed for *Bartonella henselae.*

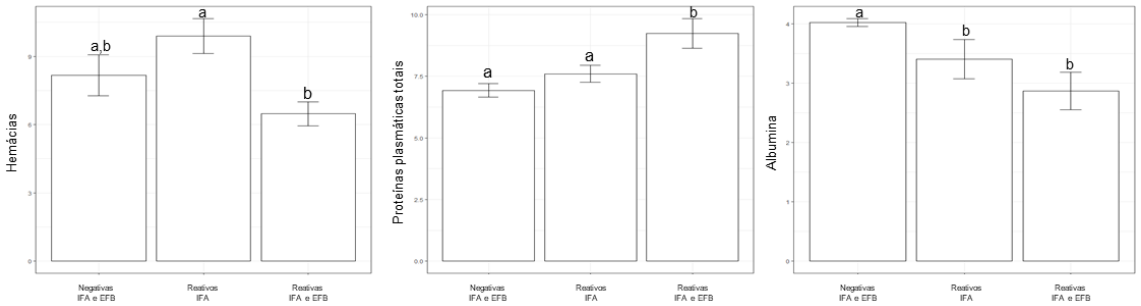
Variables	Non-reactive EFB and IFA	API Reactive	Reactive EFB and IFA
ALT (TGP)	36,38 +/- 30,26a	150,93 +/- 108,10a	44,43 +/- 29,39a
Creatinine	1,35 +/- 0,33a	1,20 +/- 0,61a	1,05 +/- 0,17a
Urea	73,76 +/- 23,61a	91,61 +/- 90,40a	65,98 +/- 21,36a
Alkaline Phosphatase	60,13 +/- 13,51a	50,73 +/- 27,83a	21,28 +/- 19,42a
Erythrocytes	8,17 +/- 1,80ab	9,89 +/- 1,34a	6,48 +/- 1,07b
Hemoglobin	12,28 +/- 2,35a	12,63 +/- 1,27a	9,20 +/- 1,49a
Haematocrit	0,40 +/- 0,09a	0,40 +/- 0,04a	0,29 +/- 0,04a
VCM	47,03 +/- 1,65a	40,20 +/- 2,21b	45,15 +/- 3,24a
CHCM	0,32 +/- 0,01a	0,32 +/- 0,01a	0,32 +/- 0,01a
Platelets	79,67 +/- 4,62a	97,00 +/- 115,97a	213,67 +/- 163,54a
Total Leukocytes	8.900 +/- 2.546a	7.933 +/- 2.996a	19.675 +/- 13.340a
Targeted	5.433 +/- 2.132a	4.694 +/- 2.034a	15.244 +/- 13.142a
Lymphocytes	2.702 +/- 1.139a	2.835 +/- 1.419a	4.179 +/- 1.266a
Monocytes	156,00 +/- 119,42a	232,00 +/- 339,10a	649,00 +/- 547,32a
Eosinophils	609,00 +/- 549,95a	64,00 +/- 56,00a	451,25 +/- 404,99a
Total plasma proteins	6,93 +/- 0,46a	7,60 +/- 0,60a	9,25 +/- 1,20b
Fibrinogen	125.00 +/- 50.00A	166.67 +/- 57.74a	150.00 +/- 57.74a
Albumin	4.03 +/-0.13A	3.41 +/- 0.66ab	2.87 +/- 0.63b

Note. Different letters on the same line identify statistical differences by GLM (*Generalized Linear Model* , in Portuguese). EFB: ELISA Feline *Bartonella*.

Red blood cells showed a small reduction in the group of animals that reacted in the EFB and in the counter-test. The animals reactive only in the API had lower Mean Corpuscular Volume (MCV) and Total Plasma Proteins (TPP), but albumin was significantly higher. The PPT was higher in all reactive samples (EFB and IFA) compared to the non-reactive animals in both tests (Figure 2).

Revista ARACÊ
ISSN: 2358-2472

Figure 2. Hematological and biochemical parameters of the felines analyzed for Bartonella henselae compared between the reactive samples for the ELISA Feline Bartonella (EFB) and indirect immunofluorescence (IFA) test.



The results showed that the three recombinant proteins (rQ1, rQ2 and rQ3) were able to identify animals with feline bartonellosis through EFB, which was also confirmed by the IFA countertest. In addition, the EFB test demonstrated high specificity, but low sensitivity.

DISCUSSION

In the Western blot *assay*, in which the recognition activity of the rQ1, rQ2 and rQ3 proteins by anti-Bartonella antibodies that could be present in the serum of naturally infected felines was evaluated, in a pool containing sera from 28 random feline patients, only rQ1 was recognized. This technique was used prior to the EFB test assays, for the initial evaluation of the antigenicity of the proteins, in order to characterize them in a qualitative and visual way. The Q1 protein was recognized by the antibodies present in the sera, indicating at that time a promising result in relation to the antigenicity potential of this chimera. This protein stood out in relation to the others in the evaluation of combined sera, as it demonstrated antibody recognition activity against *B. henselae*, which can be explained by the differentiated composition of antigenic epitopes of this chimera, since the three chimeras were developed from different fractions of the same amino acid sequence [12].

The proposed diagnostic test, ELISA *feline Bartonella* (EFB), was able to identify animals reactive to *B. henselae*, since four samples were reactive and were confirmed by the counter-test (IFA). Thus, EFB has potential as a diagnostic test for *Bartonella*, being a faster, simpler and lower cost option. However, the API identified four reactive animals, which did not react to EFB, which indicates that the proposed test has lower sensitivity. Therefore, the EFB, after adjustments in the concentrations to improve the performance of the test, can be an alternative to identify animals infected by *Bartonella*, especially in

Revista ARACÊ
ISSN: 2358-2472

animals from places with high flea infestation, such as the felines analyzed, however, it is necessary to apply a confirmatory technique, such as the IFA, since the EFB test demonstrated low sensitivity.

In these regions, the periodic diagnosis of animals, even if asymptomatic, is necessary to control the spread of the disease, as it enables the appropriate treatment and control of ectoparasites in positive animals, preventing vectors from becoming infected and continuing to spread the bacteria. Ectoparasite infestation is very relevant in the epidemiological context of *Bartonella* spp. infection. feline, and most of the cats analyzed were infested by fleas when the blood samples were collected. In addition, most of these animals were not castrated, had access to the street, contact with other animals and a history of fights, factors that significantly influence the occurrence of bacteremia by *B. henselae* [5], so the association of a correct diagnosis with appropriate treatment is the best strategy to control this infection.

The researchers cited above also identified bacterial genetic material in 47.8% of the blood samples from cats and 18.3% of *the C. felis* fleas, which demonstrates that these ectoparasites play an essential role in the transmission of *Bartonella* species to cats. However, in the present study, approximately 50% of the animals were positive, even though they were asymptomatic, reinforcing the need for a correct clinical diagnosis to control the disease, in order to prevent *Bartonella* infection in other felines, and consequently, in humans. In future research to improve the performance of the test, increasing its sensitivity, in addition, a larger number of animal sera should be analyzed, to consolidate the information regarding the sensitivity and specificity of the test.

The four reactive samples in the EFB indicate that these felines were infected with *B. henselae*, which was confirmed by the IFA and suggests that this test may be useful for the diagnosis of feline bartonellosis. However, the sensitivity of EFB for detecting IgG antibodies against *bartonella henselae* was only 50%, indicating that this method can generate false negatives. This phenomenon could be explained by the reduced concentration of antibodies present in the samples evaluated, or even due to analytical inaccuracies, since this is a test in the early stages of development.

The specificity of 100%, on the other hand, suggests that this is a method with a high capacity to identify uninfected felines, implying reduced cases of false positives, revealing the possibility of an early and accessible diagnosis. Meurer et al. [15] also evaluated the performance of the ELISA method for detecting *anti-Coxiella burnetii*

Revista ARACÊ
ISSN: 2358-2472

antibodies against the gold standard diagnostic method, also the IFA, a test that also demonstrated low sensitivity and high specificity. Therefore, even if the test still needs improvement, EFB already has great potential as a diagnostic test for *B. henselae infection.*

In these cases, EFB can be used both in individual samples and in *feline serum pools* , as a confirmatory test for *B. henselae* [16] infection, as symptomatic and asymptomatic felines are similarly likely to be infected with *Bartonella* [17]. Even so, the EFB still requires more analyses, which test different concentrations of reagents, antibodies and antigens under different conditions, and being evaluated in larger quantities of feline serum samples, from different populations, seeking its standardization and striving to improve its sensitivity as well.

Regarding hematological and biochemical analyses, felines infected with *Bartonella* generally do not present alterations in routine clinical examinations, even in the analysis of hematological and biochemical parameters [18]. The red blood cell count was lower in the animals reactive in the EFB and in the IFA counter-test, compared to the reactive animals only in the IFA. The MCV, which indicates the average size of red blood cells, may have been less smaller in the animals positive only in the IFA, while the positive ones in both tests did not differ from the negative ones, which should also be evaluated in a larger number of feline serum samples.

However, in the samples analyzed, PPT and albumin showed a difference between non-reactive and reactive samples. PPT refers to all plasma proteins, composed of albumin and globulins, and their concentrations change significantly during the systemic response to inflammation, and may be the main finding in some diseases [19]. The PPT parameter was high in all reactive samples, in both tests, in relation to non-reactive animals. Such alteration could be related to the inflammatory process triggered by infection by *B. henselae* or by concomitant infection with other infectious agents. Therefore, it would be interesting to measure this parameter in larger groups of animals evaluated for bartonellosis to better understand the relationship between this indicator and bartonellosis.

Although there are differences between these parameters, it is not possible to establish a relationship between the reaction of the animals in the EFB and the changes found, due to the small number of samples analyzed. Therefore, it is considered that the repetition of the EFB analysis in a larger number of animals together with a longitudinal analysis of hematological and biochemical parameters, in regions with cats with high

Revista ARACÊ
ISSN: 2358-2472

ectoparasite infestation, can clarify in which clinical conditions it is able to identify infected animals.

From the perspective of One Health, the well-being of man is intimately related to the animals and the environment that surround him [1]. In recent decades, the incidence of diseases in humans caused by zoonotic bacteria has increased considerably [20], and bacteria of the genus *Bartonella* represent one of the greatest contemporary challenges to One Health [7]. According to the authors, pets act as sentinels for human exposure to pathogens.

Considering that the feline population has increased considerably and the adoption of cats as companion animals has become a trend, research dedicated to zoonotic diseases is fundamental [16]. In this context, the proposed test contributes to the knowledge about bartonellosis, since the diagnosis of zoonotic pathogens from their reservoirs is one of the main ways to control these diseases [21]. Considering that zoonotic diseases are more common in populations in situations of social vulnerability, more accessible diagnostic alternatives are of great importance to predict the incidence of these diseases in these communities.

Despite the difficult standardization, a problem found in the execution of most serological tests (9), the EFB has the advantage of the specificity of the recombinant proteins used, since they are exclusive to *B. henselae*, avoiding cross-reactions, an adversity commonly faced in other diagnostic techniques [15, 22]. Negative ELISA results and positive IFA results may be indicative of the concentration of antibodies in the samples, which may be insufficient for detection by the ELISA technique, but sufficient for IFA. Therefore, future negative results found by the application of the EFB should be interpreted with caution. In addition, the development of national diagnostic tests increases the availability of these diagnostic tools and decreases the dependence on importing imported kits, which reduces their cost.

CONCLUSION

The tests performed with the chimeric proteins of *B. henselae* were able to detect the presence of antibodies against *B. henselae* both in combined samples of feline sera (*Western blot*) and in the indirect ELISA tests (EFB), with individual samples. In addition, all bartonellosis reactive samples in the proposed test were also reactive in the IFA technique, considered the gold standard in the diagnosis of bartonellosis.

Revista ARACÊ
ISSN: 2358-2472

Therefore, the recombinant proteins rQ1, rQ2, and rQ3 can be considered in the development of a diagnostic test for bartonellosis in felines. Like any innovative method, the EFB test needs improvement and standardization for its validation as a future widely disseminated commercial diagnostic test, evaluating different concentrations of reagents, antibodies and antigens, under different conditions, considering larger quantities of feline serum samples from different populations to standardize and improve the sensitivity and performance of the test proposed here.

Regarding the hematological and biochemical analyses, although there are differences between these parameters, it was not possible to establish a relationship between the reaction of the animals in the EFB and the changes found, due to the small number of samples analyzed. Therefore, studies with larger populations associating these hematological and biochemical parameters with the EFB test are necessary to elucidate in which clinical conditions it is able to identify infected animals.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

Revista ARACÊ
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REFERENCES

1. Álvarez-Fernández, A., Breitschwerdt, E. B., & Solano-Gallego, L. (2018). Bartonella infections in cats and dogs including zoonotic aspects. Parasites & Vectors, 11(1). https://doi.org/10.1186/s13071-018-3152-6

2. Angelakis, E., & Raoult, D. (2014). Pathogenicity and treatment of Bartonella infections. International Journal of Antimicrobial Agents, 44(1), 16-25. https://doi.org/10.1016/j.ijantimicag.2014.04.006

3. Ansil, B. R., Mendenhall, I. H., & Ramakrishnan, U. (2021). High prevalence and diversity of Bartonella in small mammals from the biodiverse Western Ghats. PLOS Neglected Tropical Diseases, 15(3), e0009178. https://doi.org/10.1371/journal.pntd.0009178

4. Bernal, M. K., Pereira, W. L., Ribeiro, B. C., Sarmento, V. P., & Nunes, H. M. (2023). Bartonelose: Doença de importância para a saúde pública envolvendo a tríade homem-ambiente-animal. Arquivos de Ciências Veterinárias e Zoologia UNIPAR, 26(1), 01-24. https://doi.org/10.25110/arqvet.v26i1cont-001

5. Breitschwerdt, E. B. (2017). Bartonellosis, One Health and all creatures great and small. Veterinary Dermatology, 28(1), 96-e21. https://doi.org/10.1111/vde.12413

6. BRUNT, J., GUPTILL, L., KORDICK, D., KUDRAK, S., & LAPPIN, M. (2006). American Association of Feline Practitioners 2006 Panel report on diagnosis, treatment, and prevention of Bartonella spp. infections. Journal of Feline Medicine & Surgery, 8(4), 213-226. https://doi.org/10.1016/j.jfms.2006.05.006

7. Crowther, J. R. (2009). The ELISA Guidebook. Totowa, NJ: Humana Press. https://doi.org/10.1007/978-1-60327-254-4

8. Eyer-Silva, W. D., Wutke, L. S., Paiva, A. D., Silva, G. A., Ferry, F. R., Signorini, D. J., Oliveira, J. G., & Lemos, E. R. (2020). A case of Bartonella neuroretinitis with macular star diagnosed by clinical, epidemiological, serological, and molecular data: Resolution after initiation of antimicrobial therapy. Revista da Sociedade Brasileira de Medicina Tropical, 53. https://doi.org/10.1590/0037-8682-0516-2019

9. Favacho, A. R., Roger, I., Akemi, A. K., Pessoa, J. R. A. A., Varon, A. G., Gomes, R., Godoy, D. T., Pereira, S., & Lemos, E. R. (2014). Molecular identification of Bartonella henselae in a seronegative cat scratch disease patient with AIDS in Rio de Janeiro, Brazil. Revista do Instituto de Medicina Tropical de São Paulo, 56(4), 363-365. https://doi.org/10.1590/s0036-46652014000400017

10. Ferrara, F., Di Niro, R., D'Angelo, S., Busetti, M., Marzari, R., Not, T., & Sblattero, D. (2014). Development of an enzyme-linked immunosorbent assay for Bartonella henselae infection detection. Letters in Applied Microbiology, 59(3), 253-262. https://doi.org/10.1111/lam.12286

11. Gonçalves, J. M., Cardoso, T. L., de Freitas, S. B., Woloski, R., Neto, A. C., da Silva Pinto, L., de Lemos, E. S., & Hartwig, D. D. (2022). In silico analyses and design of

Revista ARACÊ
ISSN: 2358-2472

chimeric proteins containing epitopes of Bartonella henselae antigens for the control of cat scratch disease. Applied Microbiology and Biotechnology. https://doi.org/10.1007/s00253-022-12269-3

12. Gutiérrez, R., Vayssier-Taussat, M., Buffet, J. P., & Harrus, S. (2017). Guidelines for the isolation, molecular detection, and characterization of Bartonella species. Vector-Borne and Zoonotic Diseases, 17(1), 42-50. https://doi.org/10.1089/vbz.2016.1956

13. Jost, M., Latz, A., Ballhorn, W., & Kempf, V. A. (2018). Development of a specific and sensitive enzyme-linked immunosorbent assay as an in vitro diagnostic tool for detection of Bartonella henselae antibodies in human serum. Journal of Clinical Microbiology, 56(12). https://doi.org/10.1128/jcm.01329-18

14. Raimundo, J. M., Guimarães, A., Amaro, G. M., Silva, A. T., Rodrigues, C. J., Santos, H. A., Lemos, E. R., Favacho, A. R., & Baldani, C. D. (2022). Prevalence of Bartonella species in shelter cats and their ectoparasites in southeastern Brazil. Revista Brasileira de Parasitologia Veterinária, 31(1). https://doi.org/10.1590/s1984-29612022006

15. Raimundo, J. M., Guimarães, A., Amaro, G. M., da Silva, A. T., Botelho, C. F., Massard, C. L., Lemos, E. R., Favacho, A. R., & Baldani, C. D. (2019). Molecular survey of Bartonella species in shelter cats in Rio de Janeiro: Clinical, hematological, and risk factors. American Journal of Tropical Medicine and Hygiene, 100(6), 1321-1327. https://doi.org/10.4269/ajtmh.18-0585

16. Silva, B. T., Souza, A. M., Campos, S. D., Macieira, D. D., Lemos, E. R., Favacho, A. R., & Almosny, N. R. (2019). Bartonella henselae and Bartonella clarridgeiae infection, hematological changes and associated factors in domestic cats and dogs from an Atlantic rain forest area, Brazil. Acta Tropica, 193, 163-168. https://doi.org/10.1016/j.actatropica.2019.02.026

17. Souza, A. M., Almeida, D. N., Guterres, A., Gomes, R., Favacho, A. R., Moreira, N. D., Maia, L. M., Rozental, T., Torres, R. D., Cerqueira, A. D., Lemos, E. R., & Pereira, A. N. (2010). Bartonelose: Análise molecular e sorológica em gatos do Rio de Janeiro, Brasil. Revista Brasileira de Ciências Veterinárias, 17(1), 7-11. https://doi.org/10.4322/rbcv.2014.135

18. Sayed, A. S., Alsaadawy, R. M., Ali, M. M., Abd El-Hamid, R. F., Baty, R. S., & Elmahallawy, E. K. (2022). Serological and molecular detection of Bartonella henselae in cats and humans from Egypt: Current status and zoonotic implications. Frontiers in Veterinary Science, 9. https://doi.org/10.3389/fvets.2022.859104

19. Souza, A. M., Almosny, N. R., Favacho, A. R., Almeida, D. N., Ferreira, R. F., Ferreira, E. O., Moreira, N. S., & Lemos, E. R. (2017). Bartonella spp. and hematological changes in privately owned domestic cats from Rio de Janeiro, Brazil. Journal of Infection in Developing Countries, 11(8), 591-596. https://doi.org/10.3855/jidc.8152

20. Thrall, M. A., Weiser, G., Allison, R. W., & Campbell, T. W. (2015). Hematologia e bioquímica clínica veterinária. São Paulo: Roca.

Revista ARACÊ
ISSN: 2358-2472

21. Bernal, M. K., Pereira, W. L., Ribeiro, B. C., Sarmento, V. P., & Nunes, H. M. (2023). Bartonelose: Doença de importância para a saúde pública envolvendo a tríade homem-ambiente-animal. Arquivos de Ciências Veterinárias e Zoologia UNIPAR, 26(1), 01-24. https://doi.org/10.25110/arqvet.v26i1cont-001

22. Sayed, A. S., Alsaadawy, R. M., Ali, M. M., Abd El-Hamid, R. F., Baty, R. S., & Elmahallawy, E. K. (2022). Serological and molecular detection of Bartonella henselae in cats and humans from Egypt: Current status and zoonotic implications. Frontiers in Veterinary Science, 9. https://doi.org/10.3389/fvets.2022.859104

23. Jost, M., Latz, A., Ballhorn, W., & Kempf, V. A. (2018). Development of a specific and sensitive enzyme-linked immunosorbent assay as an in vitro diagnostic tool for detection of Bartonella henselae antibodies in human serum. Journal of Clinical Microbiology, 56(12). https://doi.org/10.1128/jcm.01329-18