

UNIVERSIDADE FEDERAL DO PARANÁ

JÉSSICA SANTOS DA SILVA

SORINQUÉRITO DE *COXIELLA BURNETII* EM CÃES DE TRABALHO E SEUS
CONDUTORES NO PARANÁ: REVISÃO E ARTIGO

CURITIBA

2025

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CONDUTORES NO PARANÁ: REVISÃO E ARTIGO

Dissertação apresentada ao Programa de Pós-Graduação em Ciências Veterinárias, no Setor de Ciências Agrárias, na Universidade Federal do Paraná, como requisito parcial à obtenção do título de Mestre em Ciências Veterinárias.

Orientador: Prof. PhD Alexander Welker Biondo

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RESUMO

A *Coxiella burnetii* é uma bactéria intracelular obrigatória causadora da Febre Q em humanos e coxielose em animais, sendo assim, considerada zoonose. Seus sinais clínicos podem variar desde inespecíficos e autolimitantes até abortos ou endocardite, o que demonstra relevância para a saúde pública. As formas de infecção são variadas, sendo que a inalação possui alto risco de transmissibilidade, e sabe-se que, conforme estudos realizados em ruminantes, a transmissão por meio de carrapatos tem grande importância nessas espécies- já nos cães, são poucas as informações sobre esta forma de infecção. Os policiais dos Batalhões de Operações Especiais, bem como os cães farejadores podem estar expostos a carrapatos e diferentes ambientes durante as atividades de trabalho e também posteriormente à ação, aos policiais- sendo que o contato com os condutores é mais próximo. Estudos realizados em diversas partes do mundo demonstraram a presença do patógeno na Alemanha, Itália, Índia, Austrália e Estados Unidos, por exemplo. Considerando estes fatores e a ausência de dados relacionando a vulnerabilidade dos cães e os condutores referente à exposição dos cães ao potencial vetor e a presença de soropositividade para uma doença de saúde pública, verificou-se a necessidade de buscar saber da realidade dentro dos quarteis com canil na cidade de Curitiba. Para metodologia, foi utilizado um anticorpo IgG anti-isotiocianato de fluoresceína humana (FITC) para os testes humanos e anticorpo IgG anti-isotiocianato de fluoresceína canina (FITC) foi usado para os testes caninos. Metodologia de ensaio de imunofluorescência com avaliação pelo teste de Fisher para comparar a prevalência de soropositividade. Um total de 1/18 (5,5%) policiais e 9/30 (30,0%; IC 95% 16,66–47,88) cães foram soropositivos para anticorpos IgG anti-*C. burnetii*. A amostra humana soropositiva apresentou um título de anticorpos de fase II de 128 e era de um macho de 38 anos, sem histórico de pneumonia ou outras doenças respiratórias, e um treinador durante as operações de um cão soronegativo. Dos nove cães soropositivos, seis tinham títulos de anticorpos para ambas as fases, dois tinham apenas anticorpos anti-fase I e um tinha apenas anticorpos anti-fase II. Sete cães apresentaram títulos de 64 e dois apresentaram títulos de 32. Os nove cães soropositivos eram das raças Pastor Malinois e Bloodhound, com a idade e o método de aquisição não afetando o resultado. Os resultados mostraram maior soropositividade em cães de busca, que trabalham em uma variedade de operações, incluindo em áreas florestais, e em fêmeas, estas últimas com uma associação estatisticamente significativa, sendo 7,6 vezes mais propensas a serem soropositivas do que os cães machos. Este estudo foi o primeiro a realizar uma soroinquérito concomitante de policiais e cães de trabalho, encontrando a maior prevalência de soropositividade para febre Q entre cães militares em todo o mundo até o momento. Os resultados deste estudo devem ser considerados um alerta para exposição cruzada e transmissão de *Coxiella burnetii* entre Unidades K-9 da Polícia no Brasil e no mundo.

Palavras-chave: Soroinquérito; cães de trabalho; policiais; saúde única.

ABSTRACT

Coxiella burnetii is an obligate intracellular bacterium that causes Q fever in humans and coxiellosis in animals, and is therefore considered a zoonosis. Its clinical signs can range from nonspecific and self-limiting to abortions or endocarditis, which demonstrates its relevance to public health. The forms of infection are varied, with inhalation having a high risk of transmissibility, and it is known that, according to studies carried out in ruminants, transmission through ticks is of great importance in these species. However, in dogs, there is little information about this form of infection. Police officers in Special Operations Battalions, as well as sniffer dogs, may be exposed to ticks in different environments during work activities and also after the action, to police officers - with contact with drivers being closer. Studies carried out in various parts of the world have demonstrated the presence of the pathogen in Germany, Italy, India, Australia and the United States, for example. Considering these factors and the lack of data relating the vulnerability of dogs and handlers to a potential vector and the presence of seropositivity for a public health disease, it was necessary to seek to understand the reality within barracks with kennels in the city of Curitiba. For methodology, it was chosen to use IgG anti-human fluorescein isothiocyanate (FITC) antibody for human tests and IgG anti-canine fluorescein isothiocyanate (FITC) antibody was used for canine tests. Immunofluorescence assay methodology with evaluation by Fisher's test to compare the prevalence of seropositivity. A total of 1/18 (5.5%) police officers and 9/30 (30.0%; 95% CI 16.66–47.88) dogs were seropositive for IgG antibodies to *C. burnetii*. The seropositive human sample had a phase II antibody titre of 128 and was from a 38-year-old male with no history of pneumonia or other respiratory disease, and a handler during operations of a seronegative dog. Of the nine seropositive dogs, six had antibody titers to both phases, two had only anti-phase I antibodies, and one had only anti-phase II antibodies. Seven dogs had titers of 64 and two had titers of 32. The nine seropositive dogs were Malinois and Bloodhound breeds, with age and method of acquisition not affecting the outcome. The results showed higher seropositivity in search dogs, which work in a variety of operations, including in forest areas, and in female dogs, the latter with a statistically significant association, being 7.6 times more likely to be seropositive than male dogs. This study was the first to perform a concomitant serosurvey of police and working dogs, finding the highest prevalence of Q fever seropositivity among military dogs worldwide to date. The results of this study should be considered a warning for cross-exposure and transmission of *Coxiella burnetii* among Police K-9 Units in Brazil and worldwide.

Keywords: Serosurvey; working dogs; police officers; one health.

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LISTA DE ABREVIATURAS

BOPE	Batalhão de Operações Especiais
COVID-19	Doença infecciosa causada pelo coronavírus SARS-CoV-2
ELISA	Enzyme-linked Immunosorbent Assay
FITC	Fluorescein isothiocyanate
IFA-	Indirect Fluorescent Antibody
IgG	Imunoglobulina G
K-9	Unidades policiais com cães
mL	mililitro
UFPR	Universidade Federal do Paraná

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1 INTRODUÇÃO

Cães de trabalho são aqueles animais que participam de atividades auxiliando trabalhadores humanos em suas funções. Podem ser cães policiais participantes de buscas e apreensões de pessoas, entorpecentes, explosivos, armamentos, cadáveres, resgates, entre outros; podem ser cães de auxílio a pessoas com necessidades especiais, cães de suporte emocional, guarda, detecção de doenças, por exemplo. (Burghardt, 2003)

A bactéria intracelular obrigatória, *Coxiella burnetii*, pode causar infecção em diversas espécies de animais, especialmente mamíferos (incluindo pessoas, animais de companhia e produção), com sintomatologias diferentes entre elas. Em pessoas, a doença é chamada de Febre Q; nos animais, é denominada de coxielose (Gürtler et al, 2013).

Sua transmissão pode ser tanto pelo carrapato (que é um reservatório mas ainda não foi definido seu papel como vetor) (Kazar, 1996). Quanto por contato com material biológico contaminado, como fluidos corporais de um indivíduo doente e, a transmissão mais importante: por aerossóis. Dentre as formas conhecidas de transmissão pode-se citar o contato com produtos contaminados, como leite cru, restos de abortos, urina, fezes, com a liberação do patógeno no meio ambiente. (Damasceno et al, 2013; Palmer et al. 1983)

Dentro do organismo, a bactéria se aloja em diversos tipos celulares, como os macrófagos, monócitos, fibroblastos e também células epiteliais, formando dois tipos de variantes sendo que um é mais resistente no meio intracelular e menos resistente fora do organismo, e a outra variação é menos resistente no meio intracelular mas resiste mais fora do hospedeiro, com ação semelhante a esporos (Damasceno et al, 2013).

A infecção pela *Coxiella* sp tem caráter mundial (Damasceno et al, 2013), já sendo observadas epidemias nacionais com grande número de pessoas infectadas, como na Holanda e no Irã. Na Itália, *C. burnetii* foi isolada diretamente de carrapatos (*Rhipicephalus sanguineus*) de cães naturalmente infectados, artrópode presente em praticamente todas as regiões do Brasil (Mantovani, 1953).

Um estudo realizado por Boni e colaboradores demonstrou a presença de sorologia positiva para *Coxiella burnetii* em cães militares franceses, nas tropas alocadas na França, Guiana Francesa, Martinica, Senegal e Costa do Marfim. O mesmo estudo

comenta sobre a presença de cães positivos nos Estados Unidos, Nigéria, Suíça, Japão, Croácia, Alemanha, Itália, Nova Zelândia e Canadá. (Boni et al, 1998)

No Brasil os estudos da prevalência da bactéria se iniciaram na década de 1950, sendo que os animais de produção, especialmente os ruminantes são os mais importantes para a permanência da doença, pela sua proximidade com os carrapatos, e são os principais transmissores para a espécie humana (Guatteo, 2011). Apesar de a doença pouco conhecida no país, já foram realizados estudos nos estados do Rio de Janeiro, São Paulo, Minas Gerais, Goiás, Ceará, Alagoas, Piauí, Pernambuco, Rio Grande do Sul e Santa Catarina, e reportados casos tanto em pessoas quanto nos animais. (Mares-guia, 2014; Mioni et al, 2021; Meurer et al, 2021; França et al, 2023).

Os ruminantes infectados são os reservatórios primários e potenciais transmissores da bactéria através de restos de abortos, sêmen, leite, urina e a carne. Porém, apesar de serem portadores do patógeno, os ruminantes raramente apresentam manifestações clínicas: geralmente são assintomáticos, mas podem ter desordens reprodutivas. (Pexara et al, 2018).

O Brasil é um país com grandes rebanhos bovinos e acredita-se que estes possam ser responsáveis pelas altas taxas de prevalência observadas em inquéritos sorológicos nas pessoas (Meurer et al, 2021). Um estudo realizado entre 2008 e 2009, no Brasil, demonstrou a presença de amostras sorológicas positivas em caprinos e cães, sendo estes últimos menos estudados (Mares-Guia et al, 2014).

Quando infectados, os cães também são capazes de eliminar o agente infeccioso no momento do parto e de transmitir a bactéria por via aérea. Embora haja essa confirmação, não se sabe bem o potencial de transmissibilidade que os cães apresentam, tampouco o perfil de sororreatividade dos cães brasileiros diante de tal exposição (Angelakis, 2010). referencia

Desta forma, a Febre Q é uma doença caracterizada como zoonose ocupacional, pois o grupo considerado de maior risco de adquirir a infecção é o de profissões nas quais os trabalhadores tem contato com animais. Neste grupo estão incluídos médicos veterinários e estudantes, fazendeiros, funcionários de abatedouros e fazendas e os condutores de cães de trabalho como no caso dos policiais e militares, por exemplo. (MMWR, 2013)

A sintomatologia humana pode variar desde os casos subclínicos (sem sintomas aparentes) ou leves na manifestação aguda (com sintomas inespecíficos como febre, podendo ser tratados como uma infecção bacteriana sem especificação) e até casos

crônicos gravíssimos com ocorrência de abortos, partos prematuros, natimortos, além de fadiga crônica e endocardite (Knobel et al, 2013; Kazar, 2005). Até 5% dos casos agudos podem evoluir para a forma crônica, sendo que os sintomas podem levar até 90 dias para manifestarem. Em geral, os pacientes mais acometidos com a forma grave são aqueles em algum estado de imunossupressão (Damasceno et al, 2013). Sua importância passou a ser mais relevante desde o surto de Febre Q na Holanda, ocorrido de 2007 a 2009.

Quando infectados, os cães também são capazes de eliminar o agente infeccioso no momento do parto e de transmitir a bactéria por via aérea. Embora haja essa confirmação, não se sabe bem o potencial de transmissibilidade que os cães apresentam, tampouco o perfil de sororreatividade dos cães brasileiros diante de tal exposição (Angelakis, 2010).

A bactéria produz uma proteção de lipopolissacarídeos (LPS), que a torna muito resistente às condições normais do ambiente, dificultando sua eliminação (Gürtler et al, 2013). Por conta da gravidade das consequências quando a doença evolui para a forma crônica, faz-se necessário diagnosticar corretamente para que as ações de conscientização, prevenção, controle e, se necessário, tratamento, sejam realizadas.

O diagnóstico costuma apresentar dificuldades por conta da sintomatologia inespecífica e desconhecimento do patógeno. Quando a suspeita existe, pode-se realizar pela análise histológica, molecular ou por meio de isolamento do patógeno. A sorologia pode ser feita pela imunofluorescência indireta, ELISA ou teste de fixação do complemento. Quando a sorologia é realizada no período inicial da infecção, é possível de se observar elevação na titulação de anticorpos que se mantêm por meses. A técnica de PCR pode ser feita utilizando-se tanto o sangue quanto fragmentos de tecidos. Já para casos de endocardite, recomenda-se a técnica de histologia, apesar de não se garantir um resultado tão fidedigno. O Brasil possui poucos relatos desta enfermidade (Damasceno et al, 2013) Por conta da existência de casos subclínicos e inespecíficos, o diagnóstico muitas das vezes é prejudicado.

Cães policiais de trabalho, bem como seus condutores, podem estar expostos às zoonoses apresentadas em sua rotina. Um estudo com cães militares de trabalho que serviram no Golfo Pérsico após seus condutores retornarem das missões relatando diversas doenças de potencial zoonótico, como giardíase, parasitismo intestinal, além das doenças já relatadas na região como brucelose, leishmaniose visceral, por exemplo. (Burkman, 2001) Sendo assim, sabendo da proximidade que cães e condutores possuem, além de compartilharem o ambiente de trabalho, sugeriu-se a realização do estudo, pois

trabalhadores humanos e caninos acessam locais que podem conter os carrapatos potenciais transmissores da *Coxiella*. Desta forma, pretendeu-se realizar a detecção sorológica de anticorpos anti-*Coxiella burnetii* nesta população.

O artigo presente no próximo capítulo foi publicado em março de 2024 na revista Tropical Medicine of Infectious Diseases, no idioma inglês, e demonstra os resultados da pesquisa sorológica de *Coxiella burnetii* em cães de trabalho e seus condutores no Batalhão de Operações Especiais de Curitiba, Paraná, Brasil. O objetivo deste estudo era de identificar a presença do patógeno *Coxiella burnetii* no estado do Paraná, bem como relacionar a presença de sorologia positiva a um público que executa suas ações em áreas de risco potencial à presença de um vetor. A formatação do artigo segue o padrão solicitado pela revista. Foi o primeiro estudo de *Coxiella* realizado com cães no Brasil, e apresentou a maior positividade canina se comparada à humana. O artigo foi adicionado conforme idioma e formatação solicitados pela revista de publicação (Tropical Medicine of Infectious Diseases, 2024, 9, 78. <https://doi.org/10.3390/tropicalmed9040078>).

2 ARTIGO PUBLICADO

2.1 SOROINQUÉRITO DE *COXIELLA BURNETII* EM POLICIAIS E CÃES DE TRABALHO: RELATO DE CASO E IMPLICAÇÕES NA SAÚDE ÚNICA

Serosurvey of *Coxiella burnetii* in Police Officers and Working Dogs in Brazil: Case Report and One Health Implications

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ABSTRACT:

Background: Although the *Coxiella burnetii* infection has been investigated in dogs, its role in human transmission remains to be fully established, particularly in close and daily human–dog contact settings, such as in Police K-9 Units. **Methods:** Accordingly, this study aimed to assess anti-*C. burnetii* antibodies in clinically healthy police officers by an in-house indirect immunofluorescence assay (IFA), and working dogs by a commercial IFA Kit, from the State Special Operations Battalion, Paraná, Southern Brazil. **Results:** Overall, 1/18 (5.5%) police officers and 9/30 (30.0%; CI 95% 16.66–47.88) dogs tested seropositive to anti-*C. burnetii* IgG antibodies. **Conclusions:** To date, this is the highest prevalence of Q fever seropositivity among military dogs worldwide. Despite the low sampling rate, a statistically significant association was found between seropositivity and female dogs ($p = 0.0492$). Further studies with larger sample sizes should be conducted to establish the prevalence of Q Fever in other Brazilian K-9 Units. In summary, this study is the first to conduct a concomitant serosurvey of police officers and working dogs, and its findings should be considered a warning for cross-exposure and transmission of *Coxiella burnetii* among Police K-9 Units in Brazil and worldwide.

Keywords: One Health; public health; Q fever; zoonoses; serosurvey

2.1.1 INTRODUCTION

Despite recent reports throughout Brazil of *Coxiella burnetii* infections in animals and people [1], Q fever is still considered an undetermined disease in the country. Large livestock herds nationwide and in neighboring countries may be the cause of the high prevalence rates observed in recent human serosurveys in Brazil [2,3]. However, infected dogs may also be a potential source of human and animal infections, particularly by eliminating *C. burnetii* during delivery and infecting their owners through the air [4]. The epidemiological role of dogs in Q fever maintenance, transmission, and spread remains unclear [4].

A study of military army (not police) dogs found seropositivity rates of 34/348 (9.8%) in Southern France, 5/43 (11.6%) in Dakar, Senegal, 1/19 (5.2%) in French Guyana, and 1/12 (8.3%) in Abidjan, Ivory Coast [5]. The seroprevalence of *C. burnetii* in companion dogs has ranged widely worldwide. At the country level, companion dogs' seroprevalence rates include 5/265 (1.9%) shelters, 7/309 (2.3%) breeding, 10/328 (3.0%) households, 21/321 (6.5%) aboriginal [4], and 44/201 (21.8%) owned dogs [6,7], in the case of Australia. Data on dog serosurveys of *C. burnetii* in several countries have been gathered and presented in Table 1. In Italy, *C. burnetii* was isolated directly from *Rhipicephalus sanguineus* ticks in naturally infected dogs, the same tick species that has been identified throughout Brazil [8]. Although more than 40 tick species are currently capable of carrying *C. burnetii*, their potential for transmission to animals and humans remains unknown [4].

The Military Police—Special Operations Battalion (BOPE)—in Brazil is a group of outstanding state police officers employed in rescue and other high-risk operations, commonly using working dogs in daily routines for drugs, guns, ammunition, money, and human searching and detection [9]. Despite the possibility of acquiring ticks and tick-borne and other zoonotic diseases associated with potential infection and transmission from close and continuous human–animal relationships during such operations, no study to date has focused on working dogs and their owners. Accordingly, the present study aimed to assess and serosurvey *C. burnetii* in police officers and working dogs at the Special Operations Battalion (BOPE-PR) in Curitiba, the eighth-largest city in Brazil with approximately 1.8 million habitants.

Table 1: Worldwide results of *Coxiella burnetii* seropositivity in dogs

Location	Year	Dog Type	Sample	Positivity	Assay	Ref.
Delhi, India	1979	Stray	49	7 (14.3%)	Complement fixation	[10]
California, USA	1980	Stray	316	209 (66.1%)	Microagglutination	[11]
Nova Scotia, Canada	1985	Household	447	0	IFA	[12]
The Netherland	1987	Household	219	0	ELISA	[13]
Bologna, Italy	1992	Household	802	7 (0.9%)	ELISA	[14]
Kitaoka, Japan	1992	Stray	632	95 (15%)	IFA	[15]
Setúbal, Portugal	1995	Shelter	104	5 (4.8 %)	IFA	[16]
Southern Croatia	1995	Stray	51	6 (11.8%)	Complement fixation	[17]
Shoutheest France	1998	Military	348	34 (9.8%)	IFA	[5]
Dakar, Senegal	1998	Military	43	5 (11.6%)	IFA	[5]
French Guyana	1998	Military	19	1 (5.2%)	IFA	[5]
Abidjan, Ivory Coast	1998	Military	12	1 (8.3%)	IFA	[5]
Iraq	2011	Wild	165	9 (5.5%)	IFA	[18]
Queensland, Australia	2011	Stray	101	22 (21.8%)	IFA	[19]
Sydney, Australia	2016	Breeding	309	7 (2.3%)	IFA	[6]
Sydney, Australia	2016	Household	328	10 (3%)	IFA	[6]
Sydney, Australia	2016	Aboriginal	321	21 (6.5%)	IFA	[6]
Sydney, Australia	2016	Shelter	265	5 (1.9%)	IFA	[6]
Yangzhou, China	2016	Household	136	0	ELISA	[20]
Iran	2016	Household	182	1 (0.6%)	ELISA	[21]
South Korea	2017	Household	1023	30 (2.9%)	IFA/ELISA	[22]
Montenegro	2019	Household	259	3 (1.2%)	ELISA	[23]
Central Italy	2020	Household	516	42 (8.1%)	IFA	[24]
Queensland, Australia	2022	Pig-hunting	104	19 (18.3%)	IFA	[6]
Bangkok, Thailand	2022	Household	570	7 (1.2%)	IFA	[25]
Chile	2022	Rural	358	0	IFA	[26]
Southern Brazil	2024	Quilombo	20	1 (5%)	IFA	[27]
Southern Brazil	2024	Indigenous	406	1 (0.3%)	IFA	DN P

IFA: indirect immunofluorescence assay. DNP: data not published.

2.1.2 MATERIALS AND METHODS

This study was approved by the Human Health Ethics Committee (protocol number 3.166.749/2019), Ethics Committee of Animal Use at the Federal University of Paraná (protocol number 040/2023), and Headquarters Command of Military Police in Paraná State (protocol number 21.094.524-5).

Human and dog blood samples were collected on a single day (24 July 2021) during a requested molecular survey of saliva samples for COVID-19; SARS-CoV-2 was not detected in the samples. Policemen were sampled after signing a consent form and completing an epidemiological questionnaire, and blood samples (10 mL) were obtained by cephalic venipuncture conducted by certified nurses. The dogs were sampled after the handlers signed a consent form and completed a dog epidemiological questionnaire. Blood samples (10 mL) were obtained by jugular venipuncture conducted by certified veterinarians. Whole human and dog blood samples were placed in sterile vacuum tubes containing serum separator gel without an anticoagulant. Blood samples were kept at room temperature (25 °C) until visible clots were formed and subsequently centrifuged at 800× *g* for 5 min. The serum samples were kept at −20 °C until use.

For human diagnosis, an in-house immunofluorescence assay (IFA) kit produced in Brazil ("Ezequiel Dias Foundation, Belo Horizonte, Minas Gerais State, Brazil) containing antigens from the Argentine At12 strain, was used. This kit does not distinguish between phase I and phase II antibodies in its detection. An IgG anti-human fluorescein isothiocyanate (FITC) antibody was used for the tests. For canine diagnosis, a commercial IFA kit (SCIMEDX Corporation, Denville, NJ, USA) produced with Nine Mile antigens was used. This kit distinguishes between phase I and phase II antibodies in its detection. An IgG anti-dog fluorescein isothiocyanate (FITC) antibody was used for the tests. Serologies were performed according to the protocol described by França et al. [28]. Reactions were observed under an Olympus BX53 immunofluorescence microscope (Photonic Solutions Inc., Mississauga, ON, Canada) equipped with a 40× objective lens. For each slide tested, positive and negative controls were prepared using samples from human and canine patients previously diagnosed in our laboratory. The positive samples were subjected to serial dilutions of 1:32, 1:64, 1:128 and so on, until the final titer was reached. Previous studies in dogs and the Center for Disease Control and Prevention (CDC) manual for human diagnosis were used to define the cut-off. The cut-off point for dogs was set at 1:32, and the cut-off point for humans was set at 1:64.

Fisher's exact test was used to compare the prevalence of *C. burnetti* seropositivity among the binary variables. For variables with three or more possible answers, a chisquared test was used. The significance level was set at $p = 0.05$. All tests were performed using SAS Studio 3.81 (SAS Institute Inc., Cary, NC, USA).

2.1.3 RESULTS

Overall, 1/18 (5.5%) police officers tested by the in-house IFA and 9/30 (30.0%; CI 95% 16.66–47.88) dogs tested by a commercial IFA Kit were seropositive to anti-*C. burnetii* IgG antibodies. This is the highest prevalence of Q fever seropositivity among military dogs worldwide to date. The human seropositive sample presented a phase II antibody titer of 128 and was from a male, 38 years old, with no history of pneumonia or other respiratory

diseases, and a handler during the operations of a seronegative dog. Of the nine seropositive dogs, six had antibody titers for both phases, two had only anti-phase I antibodies, and one had only anti-phase II antibodies. Seven dogs had titers of 64 and two had titers of 32. Variables related to breed, sex, age, work specialty and method of acquisition were statistically compared with seropositivity, and the respective results are presented in Table 2.

The nine seropositive dogs were from the Malinois Shepherd and Bloodhound breeds, with the age and method of acquisition not affecting the outcome. The results showed higher seropositivity in search dogs, which work in a variety of operations, including in forest areas, and in female dogs, the latter with a statistically significant association, being 7.6 times more likely to be seropositive than male dogs.

Table 2: Demographics data of working dogs from the Special Operations Battalion of Curitiba, Brazil (2023) and their respective seropositivity for Q fever.

Variables	<i>C. burnetii</i> Positive	<i>C. burnetii</i> Negative	OR (95% CI)	<i>p</i> Value	Total Population
Breed				0.8389	
German Shepherd	0 (0.0%)	4 (100.0%)	1.0 (ref)		4
Malinois Shepherd	8 (36.4%)	14 (63.6%)	0.19 (0.00-3.97)		22
Bloodhound	1 (33.3%)	2 (66.7%)	0.18 (0.00-6.47)		3
Holland Shepherd	0 (0.0%)	1 (100.0%)	0.33 (0.00-25.4)		1
Age				0.6814	
Adults	7 (33.3%)	14 (66.7%)	1.0 (ref)		21
Senior	2 (22.2%)	7 (77.8%)	1.75 (0.28-10.7)		9
Sex				0.0492*	
Female	4 (66.7%)	2 (33.3%)	1.0 (ref)		6
Male	5 (20.8%)	19 (79.2%)	7.6 (1.06-54.1)		24
Specialty				0.1618	
Narcotics	3 (17.6%)	14 (82.4%)	1.0 (ref)		17
Explosives	1 (25.0%)	3 (75.0%)	0.64 (0.04-8.51)		4
Search	4 (66.7%)	2 (33.3%)	0.10 (0.01-0.88)		6
RPC	1 (33.3%)	2 (66.7%)	0.42 (0.02-6.40)		3
Acquisition				0.7256	
Donation	4 (26.7%)	11 (73.3%)	1.09 (0.15-7.80)		15
Purchase	2 (0.0%)	6 (75.0%)	0.64 (0.04-8.51)		8
Creation	3 (0.0%)	4 (51.1%)	0.48 (0.07-3.19)		7

* Significant *p* Value

2.1.4. DISCUSSION

The seropositivity presented in this study may provide important data on canine infection by *C. burnetii*, particularly regarding why working dogs may be more vulnerable to infection than domestic dogs, as was also observed in military dogs in the French Army [5] when compared to other dog categories worldwide (Table 1). As higher seropositivity rates in dogs are reportedly uncommon when compared with humans [1], the dogs examined in this study may have been infected by ticks during operations or at the kennels in the BOPE headquarters.

Q fever seroprevalence in domestic and shelter dogs has varied from 0 to 8.1%, whereas that in working dogs ranged from 5.2% to 18.3% (Table 1). Likewise, our results indicated that dog occupations were the likely higher associated risk factor for *C. burnetii*, as military police dogs routinely participate in search operations in forest, woody, and natural areas. A study in Australia showed that Aboriginal dogs were more likely to be infected with *C. burnetii* than domestic and shelter dogs because of higher contact with bushland and wildlife [6]. As all the dogs in this study had a history of tick infestation, contact with the natural environment and wildlife may have increased infection risk of *C. burnetii* when compared to domestic dogs living in urban settings. Due to the limited number of studies to date, the prevalence of *C. burnetii* infection among dogs in the Americas remains uncertain.

Although a *C. burnetii* infection may cause abortion, impact dog health, and impair working dog breeding, dogs in the Brazilian police battalion are mostly neutered or spayed and used for operational and not breeding purposes. Nonetheless, neutered dogs have an increased risk of being seropositive compared to intact dogs, which may occur because of the increased age of neutered dogs and greater lifetime exposure than younger dogs [7]. However, this may not be the case in the present study, in which the working dogs were all adults and seropositivity was not associated with age. In addition, dogs living within the same household have shown a higher risk of seropositivity due to shared exposure to environmental infection sources [7], as observed in working dogs in this study, who shared operational incursions at work, and rested at the police battalion kennels. In another study, dogs living in rural and agricultural regions also presented an increased risk of seropositivity, perhaps because of contaminated soil and dust from livestock farming.

In addition, marsupials in Zambia [29] and wild pigs in Australia [7] have shown *C. burnetii* seropositivity due to wildlife and nature exposure. Police dogs working in natural and rural areas that are part of the different ecosystems (Atlantic and Rain Forests, Savanah, and Mangrove swamps) of Paraná state, including the Argentinian and Paraguayan borders, should be considered vulnerable. Not surprisingly, *C. burnetii* DNA was found in 15/150 (10.0%) dogs in Zambia [29] and in 113/276 (41.0%) ticks collected from 90 dogs in South Africa, including *R. sanguineus*, *Haemaphysalis elliptica* and *Amblyomma hebraeum* tick species [30], which are also found in Brazil. Although tick-borne transmission is a major infection factor in working dogs, ticks were not found at the time of the survey due to periodic preventive programs with recently applied tick repellents.

From the One Health approach to Q fever, this study highly recommends a concomitant serosurvey of police officers and working dogs, along with environmental surveys. In such a scenario of human–animal daily sharing exposure at work, infection in dogs may impact human transmission and vice versa, as already observed for other zoonoses in vulnerable populations, such as homeless, incarcerated, indigenous, and traditional island populations [31].

Thus, the seropositive policeman in this study may serve as a warning of the airborne exposure. The working dogs sampled herein were neutered and unlikely to eliminate the agent; thus, they were no longer considered sources of infection. As a limitation, the risks of exposure of seropositive police officer in his personal activities have not been assessed, and for this reason, any evaluation cannot be carried out. Regardless, this case report should be interpreted as an alarming One Health professional risk and workers' health concern for police K-9 Units worldwide.

Seroprevalence in animals has usually been associated with the presence of nearby livestock farms or animal slaughterhouses. This is not the case in Curitiba, the city where the study was carried out. There are no slaughterhouses or livestock farms within 30 to 50 km of where the dogs live, and because it is a wooded area, it is not often windy, which would make it easier for the spores to spread. Another recurring explanation is food-borne infections, mainly due to the consumption of raw meat [32], but these animals are fed exclusively with dog food, according to the officers. Work operations are sometimes carried out in natural areas and contact with wild species and their ticks may explain the exposure. Among the mammals belonging to the local biodiversity are bats, skunks, and armadillos, and *C. burnetii* infections have already been described in bats and marsupials in Brazil [33]. In the operations, the animals are prevented from having physical contact with other species, but the role of wild animals in dispersing the bacteria in the environment is still unknown. In addition, it has been observed that the control of ectoparasites is inefficient, since the purchase of antiparasitic drugs is made through public financial transfers, which does not occur periodically. Contact with ticks may explain the high seropositivity of the group [34]. For most researchers, the potential for transmission from dogs to humans is low [35]. In addition to cattle, dogs and cats that are in close contact with humans are important potential reservoirs of *C. burnetii* during urban outbreaks of Q fever. Some cases of human infection have already been described as having dogs and cats as sources of infection; however, several pets have been found to be positive in investigations of human clusters, without these animals being incriminated in the transmission to humans [33].

Despite the reported transmission through contact with postpartum vaginal fluids, the role of dogs in the human Q fever cycle remains uncertain because this infection route has rarely been associated with human infections [36]. A single human case report has been associated with dog infection to date, during a Q fever outbreak with pneumonia that impacted three family members twelve days after exposure to an infected whelping dog, whose entire litter, sadly, did not survive [37]. Thus, further studies should be conducted on the reproductive kennels of the seropositive dog breeds found by this study, thus ruling out *C. burnetii* contact prior to admission to the Police K-9 Units.

Although a 128-cut-off point has been established to determine acute illness in people with fever [38], the same titer of the seropositive policeman examined in this study was designed as a serosurvey of clinically healthy individuals. However, even in asymptomatic cases, *C. burnetii* may remain in the body for years and cause serious complications, such as endocarditis and hepatitis [39–41]. The two dogs seropositive only for anti-phase I antibodies may have shown an infection of more than six months, where phase II antibodies may decline and end, while phase I antibodies may increase and remain stable, with *C. burnetii* persisting over time [41].

Finally, this study found that female dogs were statistically more seropositive than male dogs, and a trend of dogs specializing in search operations being more exposed than others. Dogs bred and used from a young age in operations had a tendency of higher seropositivity than dogs acquired through purchase or donation. As dogs become at risk of

infection at the time of delivery, or in cases of miscarriage and during lifetime exposure [7,38], the results of the study have corroborated higher exposure in females, search dogs, and long-exposed working dogs.

One limitation of the present study was the relatively low number of samples, which was caused by the one single sampling. In addition, part of the officers and dogs were out due to statewide duties. Further studies should be conducted with a higher number of samples and in different populations to fully establish the exact impact of *C. burnetii* in police officers and working dogs.

Human and dog blood samples were collected on a single day (24 July 2021) during a requested molecular survey of saliva samples for COVID-19; SARS-CoV-2 was not detected in the samples.

However, serological testing may not be a reliable method for determining whether specific animals are potential sources of *C. burnetii* transmission to humans. In this study, dog seropositivity to *C. burnetii* may only indicate previous exposure with no precise time of exposure or whether the exposure resulted in a clinical or subclinical disease.

2.1.5 CONCLUSIONS

This study was the first to undertake a concomitant serosurvey of police officers and working dogs, with 1/18 (5.5%) police officers and 9/30 (30.0%) dogs seropositive for anti-*C. burnetii* IgG antibodies, which is the highest prevalence of Q fever seropositivity among military dogs worldwide to date. Despite the low sampling rate, a statistically significant association was found between seropositivity and female dogs ($p = 0.0492$). The results of this study should be considered a warning for cross-exposure and transmission of *Coxiella burnetii* among Police K-9 Units in Brazil and worldwide.

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Institutional Review Board Statement: The study was conducted in accordance with the Declaration of Helsinki, and was approved by the Human Health Ethics Committee (protocol number 3.166.749/2019), Ethics Committee of Animal Use at the Federal University of Paraná (protocol number 040/2023), and Headquarters Command of Military Police in Paraná State (protocol number 21.094.524-5).

Informed Consent Statement: Written informed consent has been obtained from the patient(s) to publish this paper.

Data Availability Statement: Data are contained within the article.

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