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Vancomycin-resistant *Enterococcus* (VRE) isolates from dogs and cats in veterinary hospitals in Brazil

Ricardo Antonio Pilegi Sfasciotte^{1*}, Leandro Parussolo², Fernanda Danielle Melo¹, Mateus Felipe Schneider¹, Ubirajara Maciel da Costa¹, David Germano Gonçalves Schwarz¹, Fabiano Zanini Salbego¹ and Sandra Maria Ferraz¹

Abstract

Background In veterinary medicine, particularly concerning dogs and cats, there is limited data regarding vancomycin-resistant *Enterococcus* (VRE). However, multidrug-resistant *Enterococcus* is frequently identified, raising concerns about the potential for spreading these resistant microorganisms to humans due to their zoonotic nature. This study aimed to identify VRE colonizing animals admitted to a veterinary hospital and to determine the presence of the major resistance genes responsible for vancomycin resistance.

Results *Enterococcus* was found to carry the *vanA* gene in 52.54% of cases, the *vanB* gene in 23.73%, the *vanC* gene in 20.34%, and the *vanE* gene in 3.39%. The antimicrobials with the lowest resistance were chloramphenicol (5.08%) and ampicillin (6.78%). In contrast, the highest resistance was observed with enrofloxacin (79.66%), rifampicin (67.80%), and ciprofloxacin (61.02%). Seven *Enterococcus* isolates showed resistance to vancomycin as well as high-level aminoglycoside resistance (HLAR).

Conclusion A total of 46 animals were found to be colonized by VRE, of which 16 were healthy. The *vanA* gene was the most commonly isolated (52.54%), followed by *vanB* (23.73%), *vanC* (20.34%), and *vanE* (3.39%). This represents the first report of the *vanE* gene being identified in a dog in Brazil. Among the 59 *Enterococcus* isolates, 84.75% ($n = 50$) were found to be multidrug resistant. The colonization of VRE and *Enterococcus* HLAR in dogs and cats poses a public health concern, as it increases the risk of environmental dissemination and has implications for One Health.

Keywords VRE, Small animals, Multidrug resistance, HLAR, Antimicrobial

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Cross-border surveillance of sheep and goat rabies in Brazil

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ABSTRACT

Since Brazil has the largest territory in South America and borders 10 other countries, rabies control is strategic to prevent cross-border spread. However, prevention and control of rabies in small ruminants is neglected. The present study evaluated the spatiotemporal distribution and temporal trends of rabies in small ruminants in Brazil between 2005 and 2023. Official data on rabies case notifications and the population density of goats and sheep from the Brazilian states were used. Descriptive epidemiology and temporal analyses of high-risk clusters and trends were conducted. Rabies cases were reported in all states, except for the Federal District, Roraima, Amapá, Amazonas, and Rondônia. In sheep, 174 cases were reported, with an emphasis on Paraná (25.29 %), being highest. There were 64 cases in goats, with a highlight on Bahia (37.50 %) and Maranhão (18.75 %). However, Espírito Santo presented the highest incidence risk (IR) for goats and sheep. The highest peaks in IR occurred in 2005, 2006 and 2013. A temporal trend of decreasing goat cases was observed in northeastern Brazil from 2005 to 2023. In sheep, the Northeast region showed a downward trend in rabies cases. Three high-risk clusters were identified: the primary cluster for goats occurred in 2006, and for sheep, between 2005 and 2013. Rabies in small ruminants occurs across all Brazilian regions, with high-risk areas in the Northeast, Southeast, and South, as well as a risk of cross-border transmission. These findings support animal health authorities in strengthening rabies control for small ruminants and reducing the risk of transboundary spread.

1. Introduction

Transboundary diseases are a global threat that can impact herds of various animal species, damaging the economies of both developed countries with high technological production standards and underdeveloped countries that rely on herds primarily for subsistence [43,48]. Among animal species, small ruminants have varying degrees of socioeconomic, environmental, and cultural importance depending on the country. In Brazil, however, these species are traditionally raised in extensive pasture systems with low technological input, primarily in less productive regions compared to those used for cattle production [51]. In South America, Brazil has the largest number of goat and sheep herds, standing out as the leading producer of sheep meat and the second-largest producer of goat meat [52], with a herd size of

approximately 12 million goats and 21 million sheep [24]. The breeding of these animals has developed in a sectorized manner within the country, particularly in the southern region, where wool production is prominent, and in the northeastern region, where herds are primarily raised for family subsistence [52]. However, Brazil features regions with advanced technology for small ruminant production alongside others with lower quality and productivity, necessitating different control and surveillance strategies to manage the transmission of infectious agents between herds [51].

Among the mandatory surveillance and notification diseases for small ruminants in Brazil, rabies remains relatively neglected. The rabies virus (RABV) belongs to the *Rhabdoviridae* family and the *Lyssavirus* genus [26]. It comprises seven genotypes distributed across different continents, but only genotype 1 (RABV) is present in Latin

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Phenotypic and Genotypic of Antimicrobial Resistance Profile of *Staphylococcus* spp. Isolated from Raw Milk and Artisanal Cheese

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Abstract

Staphylococcus aureus and a few species of coagulase negative are frequently associated with food poisoning. Raw milk and dairy products are among the foods usually associated with outbreaks due to staphylococcal intoxication. This study aimed to determine phenotypic and genotypic antimicrobial resistance profiles to beta-lactam drugs in *Staphylococcus* coagulase positive (CoPS) and negative (CoNS) isolates. A total of 58 CoPS and 45 CoNS isolates recovered from raw milk and artisanal cheese from Santa Catarina were analyzed. All isolates ($n = 103$) were subjected to antimicrobial susceptibility testing. High levels of resistance to penicillin (41% of CoPS and 31% of CoNS), amoxicillin (40% CoPS), ampicillin (36% CoPS), and sulfamethoxazole-trimethoprim (35% CoNS) were observed. Twenty six percent of the isolates (18 CoPS and 9 CoNS) exhibited multiresistance profile; which means, they were resistant to at least three different classes of the antimicrobial drugs. Detection of resistance genes (*mecA*, *mecC*, and *blaZ*) was performed using multiplex polymerase chain reaction. Twelve isolates (9 CoPS and 3 CoNS) were positive for *mecA*, whereas 10 strains (4 CoPS and 6 CoNS) were positive for *blaZ*. The detection of resistant and multidrug resistant isolates emphasizes the necessity to develop strategies to better comply with good manufacturing practices and health care guidelines.

Keywords: antimicrobial susceptibility, genotypic characterization, multidrug resistance

Introduction

Serrano artisanal cheese is a widely distributed product in the mountainous region of Santa Catarina State and is manufactured with raw milk. Small-dairy farmers produce their cheese, and many of them still commercialize their products informally. In other words, the products do not go through a rigorous inspection process, which ensures the quality and safety of the product for consumers (Ide and Benedet., 2001; Melo et al., 2013). Food safety is a public health concern since products available for commercialization might pose a risk to the consumer's health (Guimarães et al., 2012).

Consumption of unpasteurized milk and raw dairy products is usually associated with staphylococcal food poisoning in humans, and the contamination is associated with poor quality of raw materials and failures in following good manufacturing practices (Santana et al., 2010).

Staphylococcus aureus is considered one of the three most important foodborne pathogens that can cause diseases in humans (Al-Nabulsi et al., 2020). In 2024, the World Health Organization (WHO) updated and refined the prioritization of antibiotic-resistant bacterial pathogens to address the evolving challenges of antibiotic resistance. This Bacterial Priority Pathogens List covers

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Prevalence and antimicrobial susceptibility of *Streptococcus equi* isolated from horses in Santa Catarina state, Southern Brazil

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Abstract

The objective of this study was to determine the prevalence of *Streptococcus equi* (*S. equi* subsp *equi* and *S. equi* subsp *zooepidemicus*) in the state of Santa Catarina and evaluate the antimicrobial susceptibility of the isolates. For this, 420 nasal swab samples were collected from randomly selected horses. Isolation and phenotypic characterization of the bacteria were performed by sowing on 5% sheep blood agar, followed by analysis of morphotinctorial characteristics and biochemical analysis. To differentiate the main beta-hemolytic *Streptococcus* in horses, the fermentation profiles of the sugar's lactose, maltose, sorbitol, and trehalose were used, which were confirmed at the subspecies level by the PCR technique. The antimicrobial susceptibility panel was defined by the disk diffusion method, testing 13 antimicrobials from ten different classes, all regularly used in equine medical clinics, followed by the calculation of the multiple antimicrobial resistance index. Ten strains of *S. equi* were isolated, with a prevalence of 2.38% (10/420). Of the total positive samples, 3% (3/10) were confirmed as belonging to *S. equi* subsp *equi* and 70% (7/10) were confirmed as belonging to *S. zooepidemicus*. Multidrug resistance was observed in 60% (6/10) of isolates. The antimicrobial with the greatest resistance was clindamycin with 70% (7/10), followed by beta-lactams, with 40% (4/10) resistance to penicillin and 30% (3/10) to ceftiofur. The isolates were 100% (10/10) sensitive to gentamicin, chloramphenicol, levofloxacin, and vancomycin. This was the first study carried out in the state, and based on these data, it can be said that Santa Catarina has a low prevalence of *S. equi* and the presence of multi-resistant strains of *S. equi* was confirmed in the equine herd in Santa Catarina.

Keywords Strangles · Equine adenitis · Epidemiology · Multidrug resistance · Upper respiratory tract

Introduction

Brazil stands out on the global equestrian scenery, having the third largest herd of horses in the world, with approximately 6 million animals, with the highest concentration of these animals in the Southeast region [1]. Among Brazilian states, Santa Catarina has the fifteenth largest national

herd and stands out in the agricultural scenario due to its strategic position for the movement of animals through the southern region of Brazil and to neighboring countries, such as Argentina, Paraguay and Uruguay. In addition to being an important land route, the state also stands out as an international reference in the production of food of animal origin. This highlights the importance of constant vigilance regarding infectious diseases, with the state of Santa Catarina being a national reference in the control of these diseases.

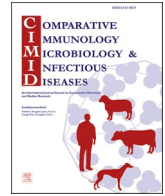
Among cross-border control diseases, those of the respiratory system are of great importance due to their rapid spread in space and producing significant economic losses when they are not previously diagnosed and treated [2]. Diseases of the respiratory system are of great importance in equine farming, producing significant economic losses if their detection and treatment are not carried out early [3]. Among the pathologies that affect the respiratory tract, strangles and strangles-like diseases stand out. Although it

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Spread analysis of glanders in the state of Piauí, northeastern Brazil

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ABSTRACT

Brazil is strategic in controlling neglected zoonoses, such as glanders, in its territory. Among the Brazilian states, Piauí is a strategic state for the spread of the disease in the country. The present study aimed to evaluate the spatial and temporal distribution of official cases of glanders in Piauí between 2015 and 2022. The glanders cases were located in the municipalities of the north and central-north mesoregions, mainly in Campo Maior, Teresina and Altos. The highest incidence risk (IR) occurred in of Altos (IR = 257.9), Sussuapara (IR = 158.4), and Teresina (IR = 157.7). A primary cluster was formed with a relative risk of 14.88 between 2019 and 2022, encompassing 34 municipalities in the north and central-north regions. In Piauí, glanders is well localized, with the potential for spread across borders. This is the first study demonstrating the distribution of reported cases of glanders in the state of Piauí.

1. Introduction

Brazil is the largest country in terms of territorial extension in South America, bordering ten countries with the exception of Chile and Ecuador. Associated with this large extension of borders, the country has the third largest herd of horses in the world, with almost 6 million heads, with the Southeast regions with 22.9% and the Northeast with 22.4% standing out as the largest herds of horses [1]. Among the Brazilian states, Piauí is the seventeenth with the largest population of horses in the country, with approximately 70 thousand horses [2], 51 thousand donkeys, and 19 thousand mules [3]. Furthermore, the northeast region where Piauí is located comprises approximately 90% of the "northeast donkeys" in the country. This species, being rustic and resistant to the semiarid conditions of the region, had great historical importance in regional development and agricultural activity, but currently there are many animals abandoned or slaughtered indiscriminately for export of their byproducts to Asia and Europe [4]. This peculiar characteristic of Piauí means that the spread of diseases in geographic space is not adequately investigated in equidae, especially in abandoned donkeys

that move between properties and come into contact with domiciled horses, which intensifies the processes of transmission of infectious agents between state and municipal borders.

Among the infectious diseases of equids with the greatest relevance to human and animal health, glanders stand out, are caused by the bacterium *Burkholderia mallei* and are characterized by pyogranulomatous lesions in the respiratory, lymphatic tract and cutaneous surface of equids [5]. Clinical signs can range from subclinical infections to acute and fulminant forms of the disease. Among the most common clinical signs are high fever, mucopurulent nasal discharge, cough, difficulty in breathing, ulcers in the nostrils and/or skin, regional lymphadenitis, conjunctivitis, lethargy, weight loss, loss of appetite, and edema in the extremities. In more serious cases, pneumonia, sepsis, abscesses in various organs, and death may occur [6,7].

In Brazil, the disease was introduced in 1811 through the import of animals from Europe and was considered eradicated in 1968. After 30 years of absence, glanders resurfaced in 1999 in Pernambuco and Alagoas, both states in the northeast region [7]. From these outbreaks, the disease spread concentrically in subsequent years, reaching nearby

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Causes of fetal death in the Flemish cattle herd in Brazil

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Abstract

Background and Aim: Flemish cattle in Brazil are on the brink of extinction and are found only in one herd in Lages, Santa Catarina State. This study aimed to uncover the reasons for the recurring abortions in the Flemish cattle herd.

Materials and Methods: Seventeen Flemish fetuses underwent postmortem examinations, with samples collected for histopathology and microbiology culture tests, polymerase chain reaction (PCR) test for *Neospora caninum*, and reverse transcription-PCR (RT-PCR) test for bovine viral diarrhea virus (BVDV) from 2015 to 2020.

Results: Of the 17 fetuses, *N. caninum* was the most common diagnosis and was found in 88% (15/17). One fetus (5.8%) had a coinfection with *N. caninum* and *Citrobacter amalonaticus*, leading to fibrinonecrotic pericarditis. All fetuses tested negative for BVDV by RT-PCR. Of the 107 dams tested by indirect immunofluorescence assay, 26 (25.2%) were anti-*N. caninum* seropositive, with 17 (65.4%) aborting and 5 (19.2%) having estrus repetition. Reverse transcription-PCR results showed that 9 (8.4%) of the serum samples collected from dams tested positive, which tested follow-up test 3 months later, indicating a BVDV transient infection. The factors that contributed to neosporosis included dogs' access to pastures and improper disposal of fetal remains, which made it easier for dogs to consume them.

Conclusion: This study warns the occurrence of *N. caninum* as a cause of reproductive disorders that can lead to abortion in the studied Flemish cattle herd.

Keywords: abortion, endangered breeds, pathology, protozoan, reproductive disorders.

Introduction

Brazil is a major player in cattle breeding, boasting an estimated herd of 218.2 million animals [1]. Despite its global prominence, the high incidence of fetal mortality due to infectious diseases and dystocia hinders the growth and productivity of the national livestock industry [2–5]. Infections are a significant cause of fetal death in cattle both in Brazil and globally [5, 6]. In Southern Brazil, there is a range of etiologies contributing to high levels of specific agents, particularly *Neospora caninum* [5–9].

Identifying the cause of miscarriages can be challenging due to tissue breakdown and multiple diagnostic techniques are often necessary to confirm if the cause is infectious or non-infectious [2, 5]. The ultimate goal of a proper diagnosis is to implement health controls in herds, ensuring the well-being of animals and people that come into contact with them [10]. In addition to financial losses from decreased reproductive efficiency in commercial herds, the presence of infectious agents can also threaten the survival of endangered breeds such as the Flemish [5, 11–13], which has a European origin and is at risk of extinction. In Brazil, the remaining pure specimens of the breed can only be found in a small herd located in the south of the country [14, 15].

Therefore, this study aimed to detect the causes of abortions through the association of histological, molecular, serological, and epidemiological analyses, identifying potentially infectious agents in the only Flemish herd in Brazil.

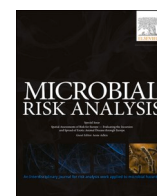
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Full length article

Spatial risk assessment of ovine Scrapie in Brazil



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ABSTRACT

Brazilian sheep farming is an ancient socioeconomic activity of great importance for maintaining income and generating family employment. Brazil is the largest producer of sheep in South America, making it a strategic country for the control of infectious diseases such as scrapie. In the present study, scrapie was officially reported in 74 cases in nine Brazilian states between 2005 and 2021. Among all Brazilian regions, the South with 54.06 % (40/74), the Midwest with 28.38 % (21/74), and the Southeast with 16.21 % (12/74) stood out with higher relative frequencies in the number of cases of the disease. Among the states, Santa Catarina presented 35.14 % of the notified cases, and the risk of incidence (IR) was 91.9 per 100,000 sheep. The years with the highest reported cases were 2012 (17 cases and IR = 2.11) and 2017 (16 cases and IR = 6.17). There was the formation of a primary cluster in the year 2017, formed only by the state of Santa Catarina, with relative risk (RRs) = 313.97, and a secondary cluster formed by the states of Mato Grosso, Mato Grosso do Sul, Goiás, Tocantins, Minas Gerais, and São Paulo in the period from 2006 to 2009, with RRs = 27.92. All of the states with reported cases shared borders, demonstrating the disease's ability to spread across state lines. Scrapie must be prevented from spreading in Brazil by implementing active surveillance measures.

1. Introduction

Brazil is one of the nineteen countries with the largest flock of sheep in the world, with more than 20 million heads. Furthermore, South America represents 5.2 % of the world's sheep herds, with Brazil being the largest producer of sheep with 31.6 % of the total, followed by Argentina with 22.3 %, Peru with 17 %, and Bolivia with 11.6 % of the total (FAO, 2022). In Brazilian territory, the state of Bahia, in the northeast region of Brazil, is the largest producer of sheep, with a total of 4247,960 head, followed by Rio Grande do Sul, in the southern region of Brazil, with 3030,419 sheep (IBGE, 2022). In this context, Brazilian sheep farming is a socioeconomic activity that has been perpetuated for generations and is characterized by commercial herds or small herds destined for subsistence, depending on the Brazilian region. Therefore, the need for knowledge of the spatial distribution of notifiable diseases

in sheep herds, such as scrapie, is fundamental to guaranteeing the quality of exported products of animal origin as well as to assist in the creation or maintenance of strategies for national programs of control and eradication.

Scrapie is an infectious disease that affects sheep and goats. It is part of the group of Transmissible Spongiform Encephalopathies (TSEs) or prion diseases and has a neurodegenerative character of fatal evolution, caused by a conformational alteration of the cellular prion protein (PrP_c), giving rise to the pathogenic form (PrP_{Sc}). The disease can present in the classic form in genetically susceptible sheep and goats or in the atypical form that was diagnosed in Norway, called "Nor98" and later observed in other parts of the world (OIE, 2018). The susceptibility of sheep to scrapie is genetically determined by the polymorphisms in codons 136 (A or V), 154 (R or H), and 171 (R, Q, or H) of the PrP gene (Woolhouse et al., 2001).

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Detection of the main multiresistant microorganisms in the environment of a teaching veterinary hospital in Brazil¹

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ABSTRACT. Sfaciotte R.A.P., Parussolo L., Melo F.D., Bordignon G., Israel N.D., Salbego F.Z., Wosiacki S.R. & Ferraz S.M. 2021. **Detection of the main multiresistant microorganisms in the environment of a teaching veterinary hospital in Brazil.** *Pesquisa Veterinária Brasileira* 41:e06706, 2021. Universidade do Estado de Santa Catarina, Av. Luiz de Camões 2090, Conta Dinheiro, Lages, SC 88520-000, Brazil. E-mail: sfaciotte@gmail.com

Contamination of the veterinary hospital environment with multiresistant pathogens endangers not only hospitalized animals, but also the workplace safety of veterinarians and nurses, animal guardians and, when in case of a teaching hospital, veterinary students. The objective of this study was to map the main points of bacterial contamination of a veterinary teaching hospital in Brazil to identify multiresistant microorganisms and their antimicrobial resistance genes. Samples were collected from 39 different locations of a veterinary school hospital which comprised a pool according to each hospital environment. In certain environments, more than one pool has been collected. All samples were collected in quadruplicates for the selective isolation of the main multiresistant microorganisms: methicillin-resistant *Staphylococcus* (MRS), vancomycin resistant *Enterococcus* (VRE), cephalosporinases and/or extended-spectrum beta-lactamase-producing Gram-negative bacteria (ESBL) and Carbapenemase-producing (CP). After isolation and identification of isolates, multiplex-PCR reactions were performed to detect the main genes for each microorganism and antimicrobial susceptibility tests with the main antibiotics used for each bacterial group according to CLSI. Of the 39 veterinary teaching hospital sites collected, all (100%) had at least one of the microorganisms surveyed, and 17.95% (n=7) of the sites were able to isolate the four pathogens. From the 94 pools collected, it was possible to isolate MRS in 81.91% (n=77), VRE in 12.77% (n=12), cephalosporinases and/or ESBL in 62.77% (n=59) and CP in 24.47% (n=23). Regarding MRS, the *mecA* gene was detected in all isolates. All isolated VREs were identified as *Enterococcus faecalis* and presented the *vanA* gene. Regarding cephalosporinases and/or ESBL, 89.83% (n=53) of the isolates presented the *bla*TEM gene, 57.63% (n=34) the *bla*OXA-1 gene, 37.29% (n=22) *bla*CTX-M gene from some group (1, 2, 9 ou 8/25) and 20.34% (n=12) the *bla*SHV gene. It was possible to identify the main microorganisms responsible for causing nosocomial infections in humans (VRE, MRS, ESBL and CP) in the veterinary hospital environment, suggesting a source of infection for professionals and students of veterinary medicine, placing a high risk for public health.

INDEX TERMS: Environment, ESBL, multiresistant, MRS, public health, Brazil.

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ANIMAL SCIENCE

Detection of virulence genes and antimicrobial susceptibility profile of *Listeria monocytogenes* isolates recovered from artisanal cheese produced in the Southern region of Brazil

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Abstract: *Listeria monocytogenes* is an opportunistic pathogen that causes listeriosis, a foodborne disease with low incidence but with high mortality rate in humans. This microorganism has been recovered from several dairy products, especially those produced with raw milk. The objective of this work was to investigate the presence of virulence genes, and also to define the antimicrobial susceptibility profile of *L. monocytogenes* isolates recovered from serrano artisanal cheese produced in Southern region of Brazil. Nine strains of *L. monocytogenes* (serotypes 1/2b and 4b) were evaluated through PCR to detect the presence of the virulence genes *hly*, *inlA*, *inlC*, *inlJ*, *actA*, *plcB* and *iap*, while antimicrobial susceptibility profile was determined via disk diffusion method. All strains exhibited the presence of the genes *hly* and *plcB*, whereas the other genes (*iap*, *actA*, *inlA*, *inlC* and *inlJ*) were only detected in eight strains. We verified that all strains were resistant to at least one antimicrobial agent and three of them showed multidrug resistance. These findings demonstrated the serrano artisanal cheese offers risks to consumers' health and point to a need of adaptations and monitoring of manufacturing process of this food, in order to prevent the dissemination of *L. monocytogenes*.

Key words: dairy products, multidrug resistance, raw milk, virulence genes.

INTRODUCTION

Listeria monocytogenes is a pathogen that causes listeriosis, a serious foodborne disease that can be fatal. The overall mortality rate is estimated in 30%, especially in those with underlying diseases and/or immunosuppressed status, elderly and fetus or neonate (Drevets & Bronze 2008, Lecuit 2007). *Listeria monocytogenes* is a concern in the food industry, since it is found in various environments, and it grows under different conditions, such as low temperatures, high salt concentrations and wide pH range

(from 4.5 to 9.0) (Guenther et al. 2009, Walker et al. 1990).

This microorganism has been recovered from several types of food, among which serrano artisanal cheese (Melo et al. 2013, Pontarolo et al. 2017), a product typically manufactured in the highland fields the South of Brazil with raw milk obtained from dairy cattle subjected to natural grazing-pasture management (Pereira et al. 2014). The raw milk contaminated by *L. monocytogenes* introduced into the food processing environment and used to manufacture unpasteurized cheese is a serious threat to human health, due to

Identification and Characterization of Multidrug-Resistant Extended-Spectrum Beta-Lactamase-Producing Bacteria from Healthy and Diseased Dogs and Cats Admitted to a Veterinary Hospital in Brazil

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The objective of this study was to identify the main extended-spectrum beta-lactamase (ESBL)-producing bacteria and to detect the frequency of the major genes responsible to trigger this resistance in hospitalized animals. We collected 106 rectal swabs from cats ($n=25$) and dogs ($n=81$) to detect ESBL-producing isolates. ESBL-positive samples were submitted to the antimicrobial susceptibility test, and polymerase chain reaction was performed to detect TEM, SHV, and CTX-M genes from different groups. We observed that 44.34% of these samples (11 cats and 36 dogs) were positive for ESBL-producing bacteria. Thirteen animals (27.66%—seven cats and six dogs) were hospitalized for elective castration (healthy animals). Only a single animal was positive for ESBL-producing bacteria at hospital admission (the animal also showed an ESBL-positive isolate after leaving the hospital), whereas 11 were positive only at the hospital discharge. Of the 73 ESBL-producing isolates, 13 were isolated from cats (8 sick and 7 healthy) and 60 from dogs (53 sick and 7 healthy). *Escherichia coli* was the major ESBL-producing bacterium isolated (53.42%), followed by *Pseudomonas aeruginosa* (15.07%), *Salmonella* sp., and *Proteus mirabilis* (5.48% each one). Antimicrobial resistance profile of ESBL-producing isolates showed that 67 isolates (91.78%) were resistant to 3 or more antibiotic classes, while 13 of them (17.81%—2 healthy cats and 11 sick dogs) were resistant to all tested antimicrobial classes. The *bla*_{TEM} gene exhibited the highest frequency in ESBL-producing isolates, followed by the *bla*_{CTX-M} group 8/25, *bla*_{CTX-M} group 1 and *bla*_{CTX-M} group 9 genes. These results are useful to assess the predominance of ESBL-producing isolates recovered from dogs and in cats in Brazil. Consequently, we draw attention to these animals, as they can act as reservoirs for these microorganisms, which are the major pathogens of nosocomial infections worldwide.

Keywords: multidrug resistance, veterinary hospital, TEM, CTX-M, small animals, ESBL

Introduction

ONE OF THE major resistance mechanisms developed by gram-negative bacteria is the hydrolysis of β -lactam ring through the action of enzymes. This process confers resistance to beta-lactam antimicrobial classes, including penicillin, cephalosporins (especially those from the third and fourth generations), and monobactams.¹ These enzymes do not hydrolyze cephamycins (cefoxitin) and carbapenems, but they can be inactivated by β -lactamase

inhibitors (clavulanic acid, sulbactam, and tazobactam).² The aforementioned enzymes are known as extended-spectrum beta-lactamases (ESBLs), which are divided into three main groups: TEM, SHV, and CTX-M.³ OXA-1 genes (found in the plasmid and integron locations of gram-negative microorganisms) are frequently associated with the worldwide spread of CTX-M-15 ESBL, conferring reduced susceptibility to cefepime (CPM), and a combination of beta-lactams and beta-lactamase inhibitors.^{4,5}

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Sepsis due to *Yersinia enterocolitica* in an aborted equine fetus: case report

Sepse por Yersinia enterocolitica em um feto equino abortado: relato de caso

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ABSTRACT

Yersinia enterocolitica is a bacterium with zoonotic potential and there are no previous records of this bacteria being isolated from aborted foals. This report aims to describe a case of sepsis due to *Y. enterocolitica* in a seven month old aborted equine. The fequinoetus was submitted to necropsy and samples of all the organs were collected for the histological exam. Samples of liver, lung, placenta, and stomach contents were collected for bacterial culture. Macroscopically, the liver was enlarged with yellowish heterogeneous color, heart with pale myocardial areas; lungs not collapsed, heavy and shiny, thickened umbilical cord covered with fibrin and pus. Histopathologically, there was moderate multifocal necrosuppurative myocarditis and thrombosis, moderate diffuse suppurative bronchopneumonia, mild multifocal fibrinonecrotic hepatitis, and moderate diffuse necrosuppurative omphalitis with intralesional bacterial myriads and thrombosis. Mild multifocal suppurative placentitis, nephritis, myositis, cystitis, and dermatitis were also observed, in addition to mild diffuse lymphoid rarefaction. The microbiological evaluation identified *Y. enterocolitica* in the liver, lung, and stomach fluid. This is the first report of sepsis due to *Y. enterocolitica* causing an abortion in a horse. This bacterium has zoonotic importance; therefore, it should be investigated in abortion in this species, serving as a differential diagnosis in reproductive disorders.

Keywords: bacterial disease, yersiniosis, zoonosis, herbivore, pathology

RESUMO

Yersinia enterocolitica é uma bactéria com potencial zoonótico, e não há informações desse agente como causa de abortamento em equinos. O objetivo deste relato é descrever um caso de sepse por *Y. enterocolitica* em um feto equino abortado aos sete meses. O feto foi submetido à necropsia, e amostras de todos os órgãos foram processadas para histopatologia. Para microbiologia, foram coletadas amostras de fígado, pulmão, placenta e conteúdo estomacal. Macroscopicamente, observou-se fígado aumentado com coloração amarelada heterogênea; coração com áreas pálidas no miocárdio; pulmões não colabados, pesados e brilhantes; e cordão umbilical espessado e recoberto por fibrina e pus. Na análise histopatológica, havia miocardite necrossuppurativa multifocal moderada e trombose, broncopneumonia supurativa difusa moderada, hepatite fibrinonecrotica multifocal discreta e onfalite necrossuppurativa difusa moderada com miríades bacterianas intralesionais e trombose. Observou-se também placentite, nefrite, miosite, cistite e dermatite supurativa multifocal discreta, além de rarefação linfóide difusa discreta. A avaliação microbiológica identificou *Y. enterocolitica* no fígado, no pulmão e no líquido estomacal. Este é o primeiro relato de sepse por *Y. enterocolitica* causando abortamento na espécie equina. Essa bactéria tem importância zoonótica, portanto deve ser investigada em casos de abortamento nessa espécie, servindo como diagnóstico diferencial em tal distúrbio reprodutivo.

Palavras-chave: doença bacteriana, yersiniose, zoonose, herbívoro, patologia

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Antimicrobial resistance evaluation of bacteria isolated from infections in small animals in the Umuarama region, Paraná¹

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ABSTRACT. Souza M.M., Bordin J.T., Pavan A.C.L., Rodrigues R.G.A., Sfaciotte R.A.P., Vignoto V.K.C., Ferrante M. & Wosiacki S.R. 2020. **Antimicrobial resistance evaluation of bacteria isolated from infections in small animals in the Umuarama region, Paraná.** *Pesquisa Veterinária Brasileira* 40(10):804-813. Departamento de Medicina Veterinária, Universidade Estadual de Maringá, Estrada da Paca s/n, Jardim São Cristóvão, Umuarama, PR 87507-190, Brazil. E-mail: srwosiacki@uem.br

Bacterial resistance is shown to be an inevitable side effect due to the excessive use of antibiotics, becoming a significant concern worldwide. Knowledge of regional bacterial resistance profiles enables the development of site-specific infection control practices, making conscious and moderate use of commercially available antibiotics. The aim of this study was the retrospective evaluation of the antimicrobial resistance profile of bacteria isolated from companion animal infections in the region of Umuarama/PR, from 2013 to 2017. This research was performed by analyzing the database belonging to the “Laboratório de Microbiologia Animal” at the “Universidade Estadual de Maringá” (UEM). *Staphylococcus* spp. represented 45.53% of the bacteria isolated from clinical infections in small animals in the period and place evaluated, followed by enterobacteria (34.04%), non-fermenting Gram-negative bacilli (NFGNB, 11.06%) and *Streptococcus/Enterococcus* (9.36%). A high number of antimicrobial resistance to antibiotics used in veterinary medicine was found. The lowest resistances associated with the best impact factor values were found for aminoglycosides, especially amikacin, chloramphenicol, and fluoroquinolones (norfloxacin and ciprofloxacin). Intermediate results were found for sulbactam-associated ampicillin, ceftriaxone, amoxicillin-clavulanic acid, and enrofloxacin. According to the number of resistant antimicrobial drugs, 64.26% (151/235) of the isolates were classified as multidrug-resistant, being 15.32% extensively resistant. Considering the resistance to antimicrobial classes, 68.94% (162/235) of the isolates were classified as multiresistant, being 19.15% extensively resistant. No bacterial strains were characterized as pan-resistant, but ten bacteria were resistant to all classes tested, with isolated susceptibility to certain drugs. Through the evaluation of resistance profiles found in the period and place studied and relevant literature, it is clear that there is a growing increase in the number of multiresistant bacteria among domestic animals which characterizes a serious risk to public health. The therapeutic arsenal is becoming increasingly diminished, and there is more difficulty in empirical drug selection, making antimicrobial susceptibility testing essential for more specific selection in antimicrobial therapy. Educational

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AGRARIAN SCIENCES

Enterotoxigenic potential of *Staphylococcus* spp. isolates recovered from raw milk and artisanal cheese

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Abstract: In this work, we investigated the phenotypic profile of *Staphylococcus* spp. isolates recovered from raw milk and artisanal cheese, and their enterotoxigenic potential through the detection of classical enterotoxin genes (*sea*, *seb*, *sec*, *sed* and *see*). A total of 104 isolates (58 coagulase-positive *Staphylococcus* – CoPS; and 46 coagulase-negative *Staphylococcus*- CoNS) were used, of which 33 were retrieved from raw milk and 71 from artisanal cheese produced in the Serrana region of Santa Catarina. Identification of CoPS was conducted via biochemical tests. Detection of the genes *sea*, *seb*, *sec*, *sed*, and *see* was carried out by multiplex PCR technique. Among the 58 CoPS analyzed, 64% were identified as *S. aureus*, 22% as *S. schleiferi* coagulans, 12% as *S. hyicus* and as 2% *S. intermedius*. In the present study was noted that 40% of CoPS isolates retrieved from milk harbored *seb* gene, while only one from artisanal cheese was positive for gene *sea*. In this study all CoNS samples investigated were negative for enterotoxins genes. The enterotoxigenic potential of CoPS, is an issue of great importance for public health. For that reason, it is necessary that cheese factories strictly follow the safety processes involved in manufacturing.

Key words: *Staphylococcus* spp., staphylococcal enterotoxins, foods, PCR.

INTRODUCTION

S. aureus is considered the most important species among *Staphylococcus* spp. due to its versatile feature to produce several endotoxins, especially the staphylococcal enterotoxins (SE). However, Valle et al. (1990) described that other coagulase-producing staphylococci exhibited the same characteristic. Later, Pereira et al. (2001) demonstrated that some coagulase-negative staphylococci (CoNS) were also able to produce toxins under laboratory conditions.

Enterotoxins are proteins of low molecular weight, which are resistant to inactivation by stomach proteases and withstand temperatures

up to 126.7°C/6.2 min. For that reason, in some cases they are able to survive in food even after thermal treatment (thermoresistant toxins) (Santana et al. 2010).

The genes encoding staphylococcal enterotoxins can be present in bacteriophages (*sea* and *sec*), plasmids (*seb*, *sed* and *selj*), staphylococcal chromosome (*seb*, *sec*, *seh*, *sei*, *selk*, *sell*, *selm*, *seln*, *selo*, *selp* and *selq*) and pathogenicity islands (*sec*, *seb*, *selk* and *selq*) (Jarraud et al. 2001, Zhang et al. 1998).

According to Sanitary Surveillance Agency (Serviço de Vigilância Sanitária) from Ministry of Health, *S. aureus* was the third most frequent pathogen associated to foodborne disease

Evaluation of minimum ripening time of artisanal mountain cheese from Santa Catarina prior to selling

Avaliação do período mínimo de maturação para comercialização do queijo artesanal serrano de Santa Catarina

Karine Andrezza Dalmina^{1*}; Leandro Parussolo²; Ricardo Antonio Pilegi Sfaciotte²; Fernanda Danielle Melo²; André Thaler Neto³; Sandra Maria Ferraz³

Highlights:

Nenhum período de maturação avaliado garantiu a inocuidade do produto final.

As propriedades apresentaram heterogeneidade nos procedimentos higiênico-sanitários.

L. monocytogenes foi isolada nos quatro períodos de maturação da mesma propriedade.

A fonte principal de contaminação do QAS pode ser pela manipulação inadequada.

Abstract

Artisanal mountain cheese (*queijo artesanal serrano*; AMS) is produced from raw milk, which makes it a suitable medium for the multiplication of pathogenic microorganisms. The ripening process involves physical, chemical and microbiological factors unfavorable to pathogen development. For this reason, the Brazilian legislation requires a minimum ripening time of 60 days. However, according to Normative Instruction (NI) no 30, of August 7, 2013, and Decree no. 9.013, of March 29, 2017, this time can be reduced provided that scientific studies show that there is no alteration in the safety of the product. This study aimed to evaluate the microbiological quality of AMS in 19 cheese factories in the Serra Catarinense region, at 14, 21, 28 and 35 days of ripening, and to determine which period would meet the microbiological limits. A total of 76 samples of AMS, 19 samples of water and 19 samples of raw milk were studied. The microorganisms were investigated following Ordinance no. 146, of March 7, 1996. The investigation and count of coliforms at 30 °C, *E. coli* and coagulase-positive *Staphylococcus* followed protocols by Silva et al. (2010), using Petrifilm™. The investigation of *Salmonella* spp. followed Silva et al. (2010), whereas the investigation of *Listeria monocytogenes* and count of mesophilic aerobes followed NI no. 30, of June 26, 2018. Water potability was evaluated by the Most Probable Number (MPN) technique, in accordance with NI no. 30/2018 and Consolidation Ordinance no. 5, of September 28, 2017. Results were tested by Analysis of Variance and Linear Regression using the SAS statistical package with significance determined at $p < 0.05$. No safe ripening period for sale could be determined. At 35 days of ripening, 14 farms still showed counts above the limit for the microorganisms described in the legislation. On one of the farms, the presence of *L. monocytogenes* was detected in the four evaluated periods, demonstrating that ripening was not efficient to eliminate the pathogen. The microbiological quality of the milk was not satisfactory, with 63.16% of the samples not conforming to the standards. As regards the potability of the water used in the cheese factories, 52.63% of the analyzed samples showed unsatisfactory results. The research revealed heterogeneity

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17 March 2020

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ASPIRATION BRONCHOPNEUMONIA BY *ACINETOBACTER BAUMANNII* IN A WILDLIFE EUROPEAN HARE (*LEPUS EUROPAEUS*) IN BRAZIL

[David G. Pereira](#), [Elizabeth Batista](#), [Thierry G. de Cristo](#), [Fábio Santiani](#), [Ricardo A. P. Sfaciote](#), [Sandra M. Ferraz](#), [Aury N de Moraes](#), [Renata A. Casagrande](#)

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Abstract

Acinetobacter baumannii is a major cause of illness in hospitalized patients and the most important and common pathogen in nosocomial outbreaks worldwide. In animals, *A. baumannii* has been associated with respiratory infections in a group of minks, leading to pneumonia and acute mortality. This report documents a case aspiration bronchopneumonia in a wild European hare caused by *A. baumannii*. A free-ranging, adult male European hare was submitted to necropsy after acute trauma due to being hit by a car. Its lungs showed consolidation with abscess in the middle and cranial lobes. Histopathologic evaluation revealed liquefactive necrosis associated with neutrophilic infiltration, cellular debris, plant material, and bacterial myriads surrounded by moderate neutrophils, macrophages, multinucleated giant cells, lymphocytes, and plasma cell inflammation. *Acinetobacter baumannii* was isolated from lung tissue.

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"ASPIRATION BRONCHOPNEUMONIA BY *ACINETOBACTER BAUMANNII* IN A WILDLIFE EUROPEAN HARE (*LEPUS EUROPAEUS*) IN BRAZIL," *Journal of Zoo and Wildlife Medicine* 51(1), 253-256, (17 March 2020). <https://doi.org/10.1638/2019-0100>

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CAPÍTULO 11

RESISTÊNCIA A ANTIMICROBIANOS EM MICRORGANISMOS RELACIONADOS COM A CADEIA PRODUTIVA DE LEITE

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Introdução

A utilização de substâncias antimicrobianas na terapêutica de doenças de cunho infeccioso revolucionou a medicina moderna, mudando o paradigma terapêutico e o prognóstico, o que torna uma das intervenções mais importantes e necessárias para o desenvolvimento de abordagens médicas complexas, como procedimentos cirúrgicos, transplante de órgãos e tratamento de pacientes com doenças complexas (MUNITA; ARIAS, 2016). O uso de substâncias antimicrobianas é comum nos sistemas de produção de leite, sobretudo no tratamento ou prevenção de doenças que acometem os rebanhos leiteiros, especialmente a mastite, doença que ameaça diretamente a lucratividade do rebanho (JAYARAO et al., 2019). Apesar do indiscutível benefício que os antimicrobianos trazem para a cadeia leiteira, potenciais efeitos na saúde pública e na indústria de laticínios podem ser observados como decorrentes do uso excessivo ou incorreto desses medicamentos. Os resíduos de antimicrobianos no leite podem causar reações alérgicas nos consumidores e distúrbios na microbiota intestinal, além de possuírem impactos carcinogênicos, mutagênicos e teratogênicos. Ainda, entre os principais problemas causados pelos resíduos de

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