Pathogens of public health concern shared by domestic and wild animals in a pluriactive farm

Patógenos importantes em saúde pública compartilhados por animais domésticos e silvestres em propriedade pluriativa pluriactive farm

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Débora Rodrigues Silveira
Mestra em Nutrição e Alimentos
Doutoranda pelo programa de Pós-Graduação em Veterinária
Universidade Federal de Pelotas
Avenida Eliseu Maciel s/nº, 354, Capão do Leão-RS, Brasil
debora.rsilveira@hotmail.com

Thamíris Pereira de Moraes
Mestra em Ciências
Doutoranda pelo programa de Pós-Graduação em Veterinária
Universidade Federal de Pelotas
Avenida Eliseu Maciel s/nº, 354, Capão do Leão-RS, Brasil
mirismoraes@hotmail.com

Kauana Kaefer
Mestra em Ciências
Residente em patologia Clínica veterinária
Universidade federal do rio grande do sul
Avenida Ipiranga, 7563, apto. 401, Porto-Alegre-RS, Brasil
kauanakaef@gmail.com

Rebeca Camargo Porto
Bacharel em Química de Alimentos
Universidade Federal de Pelotas
Avenida Eliseu Maciel s/nº 354, Capão do Leão-RS, Brasil
rebeca_porto@outlook.com

Luiz Gustavo Bach
Graduando do curso de Medicina Veterinária
Universidade Federal de Pelotas
Avenida Eliseu Maciel s/nº 354, Capão do Leão-RS, Brasil
lugubach@hotmail.com

Juliane Leite Alves
Graduanda do curso de Medicina Veterinária
Universidade Federal de Pelotas
Avenida Eliseu Maciel s/nº 354, Capão do Leão-RS, Brasil
julianemv31@gmail.com
Thaís Gonçalves Gonçalves  
Mestra em Ciências  
Secretaria de Agricultura, Pecuária, Abastecimento e Assuntos Agrários  
Prefeitura de Sant’Ana do Livramento  
Av. Dom Pedro II, 401, Faculdade, Sant’ana do Livramento-RS, Brasil  
thaais.g@hotmail.com  

Cláudio Dias Timm  
Doutor em Ciência e Tecnologia Agroindustrial  
Professor titular da faculdade de Veterinária  
Universidade Federal de Pelotas  
Avenida Eliseu Maciel s/nº 354, Capão do Leão-RS, Brasil  
timm@ufpel.edu.br  

ABSTRACT  
The objectives of this study were to identify the occurrence of pathogens of public health concern in wild and domestic animals within a pluriactive farm, to detect the presence of genes coding enterotoxins in *Staphylococcus aureus* isolates and to identify the occurrence of the same strain in different animals. Fecal samples of up to five specimens of each domestic and wild species were collected. Isolates were obtained, were realized the species identification/confirmation, detection of enterotoxigenic genes, the comparison of molecular profiles and were identified the Methicillin-resistant *S. aureus* (MRSA). Total percentage of samples from which some isolate was obtained was 7.9% (19/241). *S. aureus* was present in 7.5% (12/160) of domestic animals and in 6.2% (5/81) of wild animals. MRSA was isolated from 3.7% (6/160) of domestic and from 4.9% (4/81) of wild animals. *Yersinia enterocolitica* was isolated from 1.2% (1/160) of wild animals and *Salmonella* from 0.6% (1/160) of domestic animals. 35.3% (6/17) of *S. aureus* isolates had one of the enterotoxigenic genes. Indistinguishable and closely related isolates were found in domestic and wild animals, which confirms that there is a circulation of strains between animals or at least indicates the presence a common source of infection between them.  

Keywords: birds, *S. aureus*, MRSA, *Salmonella*, *Yersinia*, pathogen dissemination.  

RESUMO  
Os objetivos deste estudo foram identificar a ocorrência de patógenos importantes em saúde pública em animais domésticos e silvestre em uma propriedade pluriativa, detectar a presença de genes codificadores de enterotoxinas em isolados da espécie *S. aureus* e verificar a presença da mesma cepa de patógeno entre animais. Foram coletadas amostras de fezes de até cinco exemplares de cada espécie doméstica e de animais silvestres. Isolados foram obtidos, foram realizadas a identificação / confirmação de espécies, detecção de genes enterotoxigênicos, comparação de perfis moleculares e foram identificados *S. aureus* resistentes à meticilina (MRSA). O percentual total de amostras das quais foi obtido algum isolado foi 7,9% (19/241). *S. aureus* estava presente em 7,5% (12/160) dos animais domésticos e em 6,2% (5/81) dos animais silvestres. *S. aureus* resistentes à meticilina (MRSA) foi isolado de 3,7% (6/160) dos animais domésticos e de 4,9% (4/81) dos animais silvestres. Já *Y. enterocolitica* foi isolada de 1,2% (1/81) dos animais silvestres e *Salmonella* de de 0,6% (1/160) dos animais domésticos. 35,3% (6/17) dos isolados *S. aureus* possuíam algum gene enterotoxigênico. Foram encontrados isolados indistinguíveis e intimamente relacionados em animais domésticos e silvestres havendo comprovação da circulação de cepas entre animais ou indicando a presença fontes de contaminação comuns entre eles, o que confirma que há circulação de cepas entre animais ou pelo menos indica a presença de uma fonte comum de infecção entre eles.  

Palavras-chave: aves, *S. aureus*, MRSA, *Salmonella*, *Yersinia*, disseminação de patógenos.
INTRODUCTION

The pluriactivity of a farm consists of the use of more than one agricultural activity, mostly to increase the income in the farm. In the Rio Grande do Sul State, it is common that small familiar farms, as a management and profitability strategy, raise different species of domestic animals within the same territory for subsistence and/or trading (Schneider et al., 2006). Domestic animals raised within these farms are raised mainly for subsistence consumption, providing meat, milk and eggs, among other products, or as pets. When they host any microorganism that cause foodborne diseases (FBD) they may infect man, through the consumption of their products, direct contact or contamination of the environment and water (de Sá and Ferreira, 2007). They may also infect wild animals, which can act as microorganism disseminators. An important factor to be studied is the close contact between animal species, with one transmitting to the other, thus perpetuating the pathogens through their adaptation to different animals.

Bacteria of public health concern, such as S. aureus (Gomes et al., 2010), Y. enterocolitica (Silveira et al., 2018) and Salmonella (Dias et al., 2019) have already been isolated from wild and domestic animals in Brazil (Dias et al., 2019). S. aureus can infect humans (Bastos et al., 2020), and/or can produce several toxins that, once ingested, may cause food poisoning, though the microorganism can be transmitted directly from a carrier (Franco and Landgraf, 2003).

Methicillin-resistant Staphylococcus aureus (MRSA) is one of the major agents of nosocomial infections that can culminate in a variety of complications that are life-threatening (Chambers, 2001). The main strain isolated from domestic animals is predominantly related to cattle (Graveland et al., 2011). Another important strain is related to pigs and has also been isolated from cattle, poultry (Van Loo et al., 2007) and free-living wild animals (Porrero et al., 2013).

The study on the molecular profiles of Salmonella, Y. enterocolitica and S. aureus isolated from feces of domestic and wild animals represents a valuable instrument in the advance of epidemiologic knowledge of the diseases caused by these microorganisms. The identification of similar strains of these pathogens in distinct species is an important information to understand the forms of transmission of etiological agents. The polymerase chain reaction (PCR) of repetitive elements (rep-PCR) can be generated by the determination of the presence of repeated sequences in the genome through PCR of repetitive palindromic elements, that composes a molecular profile (Kudirkienė et al., 2010).

Data on pathogen carriers responsible for bacterial diseases are scarce, since it is difficult to obtain samples from wild animals. Hence, more surveys along regions are necessary, in order to determine the relevance of the bacteria transmission by wild animals to public health (Tompkins et al., 2015). The understanding on the possible interactions that promote pathogen transmission between
wild and domestic animals must also be better understood. Thus, it will be possible to define strategies to control the transmission of microorganisms of public health concern.

The objectives of this study were to identify the occurrence of \textit{S. aureus}, MRSA, \textit{Y. enterocolitica} and \textit{Salmonella} in fecal samples of wild and domestic animals within a pluriactive farm, to detect the presence of genes coding enterotoxins in \textit{S. aureus} isolates and to identify the occurrence of the same strain in different animals.

2 MATERIAL AND METHODS

Sample collection

One farm that had more than five different species of domestic animals was selected for the study. Three collection phases during spring in three consecutive years were performed, which corresponded to four successive visitations during the collection periods. Fecal samples of at least five specimens of domestic animals, randomly selected, were collected. The feces of wild animals were also collected. The species of wild animals collected varied according to the animals that were captured.

Wild birds were captured with two mist nets, of 12 meters each, positioned in strategic locations within the farm. In each visit the capture effort was of 8h, four in the end of the afternoon and the other four in the morning of the following day. After the collection of fecal samples, birds were taxonomically identified (genus and species), according to the Annotated Checklist of the Birds of Brazil by The Brazilian Ornithological Records Committee (\textit{Lista comentada das aves do Brasil pelo Comitê Brasileiro de Registros Ornitológicos}, Piacentini et al., 2015), and were discretely marked on the back with inodorous and non-toxic ink (All-Wheather, USA), in order to be identified in case of recapturing, avoiding sample duplication, and immediately released.

Fecal samples were directly obtained from the anus or the cloaca, according to the situation, with the aid of sterile swabs and dispatched to the laboratory in transport media Cary Blair (Himedia, Mumbai, India), in an isothermal container with ice.

Isolates acquisition

The determination of \textit{S. aureus} presence was carried out by directly spreading of swabs with fecal samples on Baird-Parker agar surface (Himedia, Mumbai, India) and then incubating the plate at 37°C for 48 h. Three typical and three atypical colonies of \textit{S. aureus} were inoculated on a Brain and Heart Infusion broth (BHI, Himedia) and incubated at 37°C for 24 h to perform the coagulase test. In this test, 0.3 mL of culture were inoculated in 0.3 mL of blood plasma and for 6 h of incubation at 37°C the formation of clots was monitored. The coagulase-positive isolates were submitted to PCR test to identify \textit{S. aureus}. 
The detection of *Y. enterocolitica* was carried out by streaking of the samples on MacConkey agar (MC, Himedia). After incubation at 37°C for 48 h, three lactose-negative colonies were seeded in BHI broth and, after incubation at 37°C for 24 h, motility tests were performed according to Weagant and Feng (2017). Isolates with the motility compatible with *Y. enterocolitica* were then identified through PCR test.

To assess *Salmonella*, swabs were placed into 10 mL of Buffered Peptone Water (BPW, Himedia) for pre-enrichment and remaining procedures, as recommended by US Food and Drug Administration – FDA (Andrews *et al*., 2020). Species identification was performed through PCR test.

Isolate cultures in BHI broth were mixed to 20% of glycerol to stock at -18°C. When necessary, isolates were recovered in BHI at 37°C for 24 h.

**Molecular identification**

DNA of the isolates was extracted according to Sambrook and Russel (2001). For complete cell lysis of coagulase-positive *Staphylococcus*, 100 µL of Lysostaphin solution (100 µg/mL of Lysostaphin in 20 mM of acetate buffer) was added after obtaining a pellet by centrifugation of the culture in BHI, and incubated at 37°C for 1 h.

The identification of *S. aureus* was carried out through PCR test using primers *au*-*F3* and *au-nucR*, according to a protocol previously reported by Sasaki *et al.* (2010) with modifications: the primers for the identification of *S. intermedius* and *S. hycus* were not used, and the volume of the solution was completed with water. The identification of *Y. enterocolitica* was performed through PCR test, according to Wannet *et al.* (2001), with modifications, since gene *ail* was not assessed, and primers were substituted by water. The PCR test for *Salmonella* identification was performed according to Oliveira *et al.* (2003). PCR products were stained with Blue Green (LGC Biotecnologia, Cotia, Brazil), and visualized in a 1.0% agarose gel.

**Presence of genes coding toxins in *S. aureus* isolates**

The identification of genes that coded staphylococcal toxins A (*sea*) and C (*sec*) in the *S. aureus* isolates was carried out through PCR test, as reported by Cunha *et al.* (2007), with modifications: the primers for amplification of genes *seb*, *sed* and *tst* were not used, and the volume of reaction was completed with water. For the identification of genes that coded enterotoxins B (*seb*) and D (*sed*) the PCR test was carried out as reported by Andretta (2019). PCR products, stained with Blue Green, were visualized in a 2.0% agarose gel.
Comparison of molecular profiles of the isolates

The molecular profile of the isolates of each species was assessed through the rep-PCR technique, by using primer (GTG)$_3$ (Versalovic et al., 1994). The products of rep-PCR were submitted to a 2% agarose gel electrophoresis and stained with Blue Green for the visualization of band patterns of the different amplified regions. Band profiles obtained were analyzed comparatively according to Tenover et al. (1995).

Identification of Methicillin-resistant *Staphylococcus aureus*

Strains previously confirmed as *S. aureus* were submitted to disk-diffusion test in Müeller-Hinton agar, using a 30 µg of cefoxitin disk (Skov et al., 2003) for the identification of MRSA. The results obtained were assessed according to the Clinical and laboratory Standards Institute (CLSI, 2015), which considers a microorganism as resistant when the inhibition halo has a diameter lower than 21 mm and sensitive when halos have a diameter equal to or higher than 22 mm.

This study was authorized by the Animal Experimentation Ethics Committee of the Universidade Federal de Pelotas, code CEEA n. 0978-2016. The capture of wild animals was authorized by the Chico Mendes Institute for Biodiversity Conservation (ICMBio), n. 52646-1.

3 RESULTS AND DISCUSSION

One hundred and sixty domestic animals and 77 wild birds were sampled. One of the wild birds, *Cairina moschata* (Muscovy duck) had a nest within the area of the farm’s headquarters. Moreover, fecal samples from one *Rattus rattus* (Black rat) and three *Sus scrofa* (Wild boars), captured by the farm’s owner during the execution of the survey, were collected, making a total of 241 fecal samples. The total percentage from which at least one isolate was obtained was 7.9% (19/241), with 0% (0/79) in the first collection phase, 25.4% (15/59) in the second, and 3.9% (4/103) in the third collection phase (Figure 1).
In the first phase, 58 fecal samples of domestic animals and 20 fecal samples of wild birds of nine species were collected, including samples of *Cairina moschata* and *Rattus rattus*. The microorganisms surveyed were not isolated from any of the samples.

In the second phase, 53 fecal samples of domestic animals of 11 different species and six fecal samples of wild birds of four species were collected. Eleven out of 53 (20.8%) of the domestic animals’ samples were contaminated by *S. aureus*, which were obtained from pigs (2/3), cats (2/3), chickens (2/2), dogs (2/2), turkeys (2/2), and sheep (1/1). *S. aureus* was also isolated from 66.7% (4/6) of the wild animals’ samples, which were the birds of the species *Sicalis flaveola* (1/2), *Turdus rufiventris* (1/1), *Furnarius rufus* (1/1) and *Passer domesticus* (1/1). Of the *S. aureus* identified, 53.3% (8/15) were resistant to cefoxitin and therefore classified as MRSA, in which 33.3% (5/15) were obtained from feces of domestic animals, chickens (2/2), pigs (1/3), dogs (1/2), turkeys (1/2), and 20.0% (3/15) from the wild birds *S. flaveola* (1/2), *T. rufiventris* (1/1) and *F. rufus* (1/1). *Y. enterocolitica* and *Salmonella* spp. were not isolated from any of the samples.

In the third phase, feces from 49 domestic animals of 12 different species were collected and 51 wild birds of six species were captured and sampled, in addition to samples of three *Sus scrofa*. Two (1.9%) animals were infected by *S. aureus*, one bull (1/5) and one wild bird of *S. flaveola* species (1/17). Both (100%) were classified as MRSA. One (0.1%) of the 103 animals surveyed hosted *Y. enterocolitica*, one passerine of *S. flaveola* (1/17) species. *Salmonella* was isolated in one (0.1%) out of the 103 samples, from the feces of a cat (1/4).

When the molecular profiles of the *S. aureus* isolates obtained from domestic and wild animals were compared, some of them were considered indistinguishable or closely related (Figure 2). One of
them was common to a chicken, a dog, a cat, a pig, and a bull (profile A), comprising two phases of sample collections, and the other was common to a sheep and a *F. rufus* (profile F).

Figure 2. rep-PCR electrophoresis gel of the *Staphylococcus aureus* isolates obtained from fecal samples of domestic and wild animals of a pluriactive farm. Profiles are grouped by letters according to the similarity.

As for the presence of genes coding enterotoxins, four (23.5%) out of the 17 isolates of *S. aureus* had the *sec* gene, which were obtained from cats (2/5), dogs (1/5) and chicken (1/5), and two (11.8%) had the *sed* gene, obtained from a dog (1/5) and a bird of *T. rufiventris* species (1/1), all of them during the second phase. *Sea* and *seb* genes were not present in any of the isolates.

The percentage of isolates obtained from domestic and wild animals did not differ greatly (Figure 1), except for the 66.7% of the isolates from feces of wild birds found in the second phase, which could be the consequence of the small number of wild birds captured in this phase. This is an important epidemiological association that suggests that the sources of contamination for both domestic and wild animals were equally influenced by factors that favored bacterial replication, possibly climatic or the management within the farm. On the other hand, there was a significant variation of the percentage of isolation between collection phases, which demonstrates that the level of infection in the animals varies from year to year, possibly due to climatic and management variations within the farm as well.

One of the reasons that could explain the high occurrence of pathogens in wild birds and the small number of captured birds in the second phase, is that passerines seem to serve as a reservoir and
those that were not already infected are more susceptible. Therefore, outbreaks related to enterobacteria may occur when the birds are in a poor condition, as described by Söderlund et al. (2019) in a study that reported the transmission of Salmonella by passerines.

This study demonstrated that pigs, cattle, sheep, birds, dogs, and cats raised in pluriactive farms, as well as wild birds of the species S. flaveola, T. rufiventris, F. rufus and P. domesticus could serve as reservoirs of S. aureus, being able to disseminate the pathogen through their feces in the environment. These species of both domestic and wild animals, when hosting S. aureus, constitute potential transmitters of pathogens to both humans and other animals, by direct contact through the consumption of animal products or by environmental and water contamination (de Sá and Ferreira, 2007). On the other hand, wild birds could be infected through domestic animals and humans (Daszak et al., 2000). The fact that free-living birds S. flaveola, T. rufiventris and F. rufus carried MRSA presupposes that their infection occurred directly or indirectly from domestic animals or humans, from which these strains were originated.

Isolates that were common among domestic animals (profile A) and among domestic and wild animals (profile F) were found, indicating a transmission interaction of strains between domestic and wild animals or a common source of infection. This variation among highly different carriers is possible by the fact that some strains of S. aureus have few specificity restrictions regarding the host and are able to colonize several species (Graveland et al., 2011) and continue their perpetuation. The occurrence of a common molecular profile (profile A), found both in the first and the second phases, indicates the permanence and circulation of a pathogen strain for a long period among domestic animals within the farm.

The presence of MRSA strains weakly species-specific in rural areas increase the concern regarding the dissemination among animals and the potential infection of humans. It has already been demonstrated that people that hosted MRSA when in direct contact with both production or companion animals, exhibited an increased risk of carrying the same strains of MSRA as animals (Morgan, 2008). Once MRSA infect humans, there is great difficulty in the treatment of patients due to the broad resistance of this microorganism to antibiotics.

The presence of the sec gene in 23.5% (4/17) and the sed gene in 11.8% (2/17) of S. aureus isolates indicates the potential pathogenicity of strains present in pets, production and wild animals within the farm. In a study conducted by Smyth et al. (2004), the sec gene was present in 51 out of 191 S. aureus isolates obtained from cattle (19/99), goats (18/39) and sheep (14/23), and one isolate obtained from a cat had the sed and sej genes. The presence of two cats, one dog and one chicken hosting S. aureus with the sec gene and one dog hosting S. aureus that had the sed gene suggests risks to human and animal health. Furthermore, the fact that one T. rufiventris hosted S. aureus with the sed
gene represents a potential risk for the perpetuation of the pathogen in the farm and a greater potential for the dissemination of this strain, since it was found in a free-living bird.

*S. flaveola* may carry *Y. enterocolitica* and, consequently, be a potential disseminator of these pathogens to the environment, domestic animals, men and food. *Y. enterocolitica* seem to exhibit lower transmissibility than *S. aureus* (Silveira et al., 2018). Possibly because of that, a lower occurrence of *Y. enterocolitica* was observed in our study.

The cat that hosted *Salmonella* could have been infected from passerines throughout the years. It has already been demonstrated that mass seasonal migration of passerines seems to cause outbreaks of *Salmonella Typhimurium* among cats in certain years in Sweden, probably due to predation of weakened birds. From this transmission, outbreaks can occur among humans, caused by contact with infected domestic cats or through environmental contamination (Söderlund et al., 2019). Others carriers of *Salmonella* were not found within the farm, neither in domestic nor in wild animals, that could be the source of contamination. On the other hand, pets can be infected through humans which are possible sources of infection.

### 4 CONCLUSIONS

Domestic and wild animals in a pluriactive farm can be pathogens of public health concern carriers and share the same strains, which can remain circulating among the animals of the farm for at least one year.

*S. aureus*, including MRSA, can be present in the feces of both domestic (pigs, cats, chickens, turkeys, sheep, and cattle) and wild animals (*S. flaveola, T. rufiventris, F. rufus* and *P. domesticus*) within the same pluriactive farm. Cats, dogs, chickens and *T. rufiventris* can carry potentially enterotoxigenic *S. aureus*. *S. flaveola* can be a reservoir of *Y. enterocolitica*, as well as cats can carry *Salmonella*. Considering the variety of species, both domestic and wild, that can carry bacteria of public health concern and the long time that these pathogens remain in the farm, it becomes necessary that hygienic-sanitary care measures are adopted, in order to minimize the possibility of transmission of these microorganisms and decrease the risk of infection for humans, domestic and wild animals and as well as environmental contamination.

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