UNIVERSIDADE FEDERAL DA PARAÍBA

CENTRO DE CIÊNCIAS AGRÁRIAS

PROGRAMA DE PÓS-GRADUAÇÃO EM CIÊNCIA ANIMAL

MASTITE ESTAFILOCÓCICA POR MRSA EM PEQUENOS RUMINANTES NO ESTADO DE OHIO – EUA

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Médico veterinário

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Guilherme Santana de Moura

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EPÍGRAFE

Assim, não desistamos de fazer o que é bom,

pois colheremos no tempo devido,

se não desanimarmos.

Gálatas 6:9

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Aos meus pais e a minha irmã.

Que são, sempre foram e sempre serão a minha base.

Aos meus familiares e amigos.

Dedico.

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CoNS – Coagulase Negative Staphylococcus

HA-MRSA – Human Associated *Methicillin Resistant Staphylococcus aureus*

LA-MRSA – Livestock Associated *Methicillin Resistant Staphylococcus aureus*

MLST – Multilocus Sequence Typing

MRSA – *Methicillin Resistant Staphylococcus aureus*

PCR – Polimerase Chain Reaction

ST – Sequence type

USA – United States of America

MASTITE ESTAFILOCÓCICA POR MRSA EM PEQUENOS RUMINANTES NO ESTADO DE OHIO – USA

RESUMO GERAL- A mastite tem um grande impacto na economia bem como no bem-estar animal na produção de ovinos e caprinos. Embora vários microrganismos possam causar mastite em pequenos ruminantes, o *Staphylococcus aureus* é um dos agentes mais importantes na sua epidemiologia. Uma porcentagem crescente dos isolados que são originários das mais diferentes fontes, apresentam resistencia à meticilina (Methicillin Resistent *Staphylococcus aureus* - MRSA) podendo provocar doenças graves em seres humanos e animais. MRSA associado a animais de produção (LA-MRSA) são cepas frequentemente isoladas de animais, veterinários e pessoas que vivem e trabalham em sistemas de criação animal, sendo a cepa ST398 a mais frequentemente identificada inclusive em infecções humanas. Entretanto, outras cepas como ST133 estão comumente associadas a problemas sanitários em rebanhos leiteiros. O presente trabalho aborda casos de mastite causadas por cepas de MRSA em rebanhos caprinos e ovinos em Ohio – EUA. Um caso de mastite supurativa em uma ovelha causada por MRSA ST398, o primeiro identificado em um rebanho ovino nos Estados Unidos, também é relatado. Uma ovelha com febre, linfonodos mamários reativos, magreza, fraqueza muscular e mastite em ambas metades do úbere foi examinada. Um dos tetos tinha uma fístula drenando pus. Uma amostra da secreção purulenta foi colhida e enviada para análise. Testes fenotípicos e genotípicos foram realizados. A amostra foi nucA e mecA positiva confirmando geneticamente o S. aureus resistente à meticilina. MLST revelou que o isolado testado pertencia a linhagem ST398. Este é o primeiro relato de uma mastite em uma ovelha causada por MRSA nos EUA. A identificação de MRSA ST398 é importante e merece mais investigações epidemiológicas sobre o patógeno em sistemas de produção animal. O segundo estudo teve como objetivo investigar a ocorrência de S. aureus resistente à meticilina (MRSA) associado a mastites em rebanhos caprinos no Estado de Ohio, EUA. Um total de 120 amostras de leite e 120 amostras de suabe de teto foram colhidas de cinco fazendas. Depois de

isolamento convencional e da caracterização fenotípica das colônias, realizou-se a caracterização molecular através de PCR para os genes nuc e mecA para confirmação do MRSA e os complexos clonais determinadas por MLST. Quinze (6,2%) amostras positivas de S. aureus foram encontradas neste estudo; nove de amostras de leite e cinco de amostras dos tetos. Quatro (2%) isolados de MRSA foram detectados e pertenciam aos complexos clonais ST133 e ST5. Três isolados coagulase negativa (1,25%) possuiam o gene mecA. Este estudo indica que cepas de MRSA pertencentes ao complexo clonal 133 podem estar associadas a infecções intramamárias em rebanhos caprinos nos EUA e a identificação do MRSA ST5 é de especial preocupação, uma vez que é um importante patógeno para os humanos.

Palavras-chave: Mastite em pequenos ruminantes, LA-MRSA, ST398

STAPHYLOCOCCAL MASTITIS BY MRSA IN SMALL RUMINANTS IN OHIO STATE – USA

ABSTRACT – Mastitis has a large impact on the economy as well as on animal welfare in sheep and goat production. While several microorganisms can cause mastitis in small ruminants, *Staphylococcus aureus* is one of the most important pathogen. An increasing percentage strains showing resistance against methicillin (Methicillin Resistent *Staphylococcus aureus* - MRSA) have been recovered from different sources and can potentially cause severe illness in humans and animals. Livestock associated MRSA (LA-MRSA) are strains often isolated from animals, veterinarians and people who are in contact to food animals and the sequence type 398 is the frequent LA-MRSA strain associated to human infections. This paper discusses mastitis cases caused by MRSA strains in goats and sheep herds in Ohio - USA. One sheep showing fever, reactive mammary lymphnodes, thinness, muscle weakness and mastitis in both mammary glands was examined. One teat had a fistula draining pus. A sample of purulent discharge was collected and sent for analisys. Phenotypical and genotypical tests were performed. Sample was *nuc* and *mecA* positive confirming genetically the Methicillin resistant *S. aureus*. MLST revealed that the tested MRSA isolate belonged to the clonal lineage ST398. This is the first MRSA report causing mastitis in sheep in USA. Mostly important, the identification of ST398 warrants further epidemiological investigations about epidemiology of this pathogen in animal production systems. The second study aimed to investigate occurrence of Methicillin Resistant *S. aureus* (MRSA) associated with intramammary infections in goat herds in Ohio State, USA. A total of 120 milk samples and 120 teat-swab samples were collected from five farms. After conventional isolating and phenotypic characterization of colonies, molecular characterization was also performed through PCR for *nuc* and *mecA* genes for MRSA confirmation and clonal complexes determined by MLST. Fifteen (6.2%) positive *S. aureus* samples were found in this study; nine from milk and five from teat skin samples. Four (2%) MRSA isolates were detected and belonged to clonal complexes ST133 and ST5. Three (1.25%) coagulase-negative isolates were shown to harbor the mecA gene. This study indicates

that MRSA belonging to clonal complex 133 can be associated with intrammamary infections in goat herds in US and the identification of the MRSA ST5 is of special concern, since it's an important pathogen to humans.

Key-words: Small ruminant mastitis, LA-MRSA, ST398

CONSIDERAÇÕES GERAIS

A mastite tem um grande impacto econômico, bem como no bem-estar de caprinos e ovinos em sistemas de produção. Em ovelhas e cabras, ela é causada principalmente pela infecção por bactérias gram-positivas, incluindo estafilococos, estreptococos e enterococos. Bactérias gram-negativas, principalmente enterobactérias, também podem causar mastite nestes animais, embora com ocorrência significativamente menor do que em vacas (Bergonier et al. 2003). Micoplasmas são importantes causadores de mastite em pequenos ruminantes mas, por determinarem também lesões sistêmicas associadas a diversos sinais como claudicação, ceratoconjuntivite e problemas respiratórios, não são considerados agentes causadores primários da mastite (Pisanu et al. 2015). Embora vários microrganismos possam causar mastite em pequenos rumianantes, *Staphylococcus aureus* (*S. aureus*) é um dos agentes mais importantes na sua epidemiologia (Ciftci et al. 2009), tanto nas formas clínica ou subclínica. Rotineiramente, estes patógenos causadores de mastite são isolados e classificados quanto ao gênero e espécie de forma generalista. No entanto, o *S. aureus* possui uma vasta gama de genótipos com características patogênicas e epidemiológicas variadas (Pisanu et al. 2015).

S. aureus produz uma grande variedade de fatores de virulência, os quais originam-se da parede bacteriana ou secretados. Dentre os principais fatores de virulência incluem-se toxinas superantigênicas, enzimas, citotoxinas, exotoxinas e toxinas esfoliativas, cuja função principal é transformar os componentes do hospedeiro em nutrientes para o crescimento bacteriano, modulando a patogenicidade, sintomas e severidade das infecções (Santana et al. 2010). Embora haja pouca dúvida de que o principal e mais importante reservatório de *S. aureus* é a própria glândula mamária infectada destes animais, estudos mais amplos são necessários para aumentar o conhecimento dos autores sobre outros possíveis reservatórios, as vias de transmissão, a dinâmica da infecção, os fatores de risco, e as associações entre os isolados clonais (Vautor et al. 2005). Uma vez que o *S. aureus* é considerado um agente patogénico oportunista, é possível que certos clones são mais propensos a aderir e colonizar o úbere das ovelhas por causa da presença de determinados

fatores de virulência, aumentando o seu potencial para a adesão e colonização em comparação a outros clones (Zastempowska; Lassa, 2012). Muitos fatores de virulência foram identificados no genoma *S. aureus*, as diferenças na patogenicidade entre os isolados de campo permanecem em grande parte desconhecidos (Harrison et al. 2013).

A abreviação MRSA orifina-se do termo inglês “Methicillin Resistant *Staphylococcus aureus*” e é utilizada para designar as cepas de *S. aureus* não responsivas ao tratamento com antibióticos β-lactâmicos, incluindo a meticilina ou oxacilina. Caracterizam-se por possuir o gene *mecA* ou demonstram uma concentração inibitória mínima (CIM) à oxacilina maior do que 4 mg/L (Cerqueira, 2013). Entretanto, alguns isolados clínicos podem ser *mecA*-positivos mas susceptíveis à oxacilina. O gene *mecA* é responsável pela codificação da proteína de ligação à penicilina (PBP2a) que funciona como um alvo alternativo resistente à inibição pelo antibiótico, permitindo a formação da camada de peptídeoglicano da parede celular e impedindo a morte bacteriana (Velasco et al. 2014). As infecções humanas causadas por MRSA são relativamente comuns e vastamente reportadas, principalmente em ambientes hospitalares. Fatores de risco para contaminação nosocomial por MRSA incluem hospitalização prévia, período prolongado de hospitalização, uso prévio de antibiótico, entre outros.

Contudo, o crescimento no número de registros de infecções comunitárias graves, especificamente em indivíduos que não foram hospitalizados e nem estiveram em contato com profissionais da saúde, ou mesmo com doentes colonizados, são preocupantes e sugerem a existência de determinantes epidemiológicos associados à emergência de MRSA fora dos hospitais. O uso indiscriminado de drogas antimicrobianas na pecuária é considerado um fator importante na seleção e disseminação de MRSA (Vyletelova et al. 2011). O contato direto do homem com os animais, a exposição às fontes ambientais contaminadas com resíduos da produção pecuária e, principalmente, os alimentos de origem animal, constituem meios de transferência de MRSA para humanos (Hetem et al. 2013). Assim, mesmo que MRSA não seja considerado um patógeno alimentar, sua presença em

alimentos representa uma via de disseminação da bactéria para populações humanas.

As informações epidemiológicas disponíveis corroboram essa hipótese. Algumas cepas de MRSA com elevado potencial zoonótico foram identificadas primeiramente em suínos (2005) e em seres humanos que trabalhavam em suinoculturas na Holanda e na França. Dessa forma, foram designadas MRSA associado a animais de produção ("livestock-associated MRSA", LA-MRSA) (Pantosti, 2012) e pertencem ao complexo clonal (CC) 398. Posteriormente, cepas idênticas foram encontradas em animais saudáveis de muitas outras espécies, tais como equinos, bovinos e frangos.

LA-MRSA é definido com base em dados epidemiológicos e genéticos, como o tipo de SCCmec presente e o fato de ser isolado de animais, veterinários e pessoas que vivem e trabalham em explorações (Concorde, 2013). Estas cepas LA-MRSA têm algumas características típicas como resistência à clivagem pela enzima de restrição *SmaI* (enzima de restrição normalmente usada para tipagem de *S. aureus* por PFGE), tendo por isso mesmo sido classificadas, inicialmente, como não tipáveis por PFGE (Nemeghaire et al. 2014); pertencem sobretudo ao CC398, sendo a ST398 a mais frequentemente identificada; têm SCCmec do tipo IV ou V, sendo este último o mais frequente e a maioria das estirpes não têm toxinas, como a PVL (Pantosti, 2012). Um estudo realizado para averiguar a prevalência de MRSA em suinoculturas detectou este agente na maioria dos países Europeus (17 de 24 participantes), tendo também verificado associação positiva entre prevalência e densidade animal (Authority, 2009). Estas estirpes LA-MRSA tem sido também detetadas em suínos no Canada e EUA (Smith et al. 2013).

O clone CC398 já foi detectado em outras espécies, como caninos, equinos, bovinos e homem, principalmente em pessoas com contato próximo aos suínos, como veterinários e trabalhadores rurais (Nienhoff et al. 2009 e Smith et al. 2013). Atualmente, sabe-se que o LA-MRSA CC398 teve origem no ser humano como *S. aureus* sensível a meticilina, tendo-se disseminado, posteriormente, para os animais de produção onde adquiriu resistência à meticilina e tetraciclinas e perdeu o fago que contém os genes responsáveis

por evasão ao sistema imunológico (“immune evasion cluster”, IEC) cruciais na adaptação ao hospedeiro humano (Smith et al. 2013).

Dados sugerem que esta passagem do ser humano para os animais foi acompanhada por uma diminuição da capacidade de colonização, transmissão e virulência, nesse hospedeiro, contudo este clone tem sido identificado em infeções humanas (Neyra et al. 2014).

De uma maneira geral, as cepas de MRSA identificadas em animais de companhia e cavalos são diferentes das obtidas de animais de produção. Em animais de companhia e cavalos, as cepas são normalmente similares aos clones MRSA humanos, enquanto nos animais de produção as cepas parecem pertencer a clones específicos adaptados aos animais (Pantosti, 2012). Essas conclusões derivam de investigação epidemiológica molecular, através do uso de técnicas de subtipificação, capazes de fornecer informações sobre o grau de clonalidade dos isolados. (Vautor et al. 2005).

Diferentemente do que ocorre em bovinos, pouca informação está disponível sobre a epidemiologia de *S. aureus*, especialmente MRSA, em pequenos ruminantes. O presente estudo objetivou, através da aplicação da epidemiologia molecular, gerar conhecimento sobre a ocorrência MRSA associado a mastite em ovelhas e cabras

CAPÍTULO 1

Livestock-Associated MRSA ST398 in a sheep herd - USA.

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Livestock associated MRSA (LA-MRSA), particularly ST 398, poses an increasing risk to public health. Although MRSA has been identified as a mastitis causative agent in dairy cattle, this is the first MRSA ST398 report causing mastitis in sheep in USA.

An investigation on lambs growth impairment in a sheep herd, comprised of 300 ewes and 85 replacement lambs which are Polypay crossed with a Suffolk sire was conducted in Ohio – USA. The lambs are raised on grass, in intensive grazing management on pure alfalfa stands.

A total of 25 lambs were examined, weighed and followed-up for two weeks. Parasitic screening test was performed by McMaster for fecal egg counting. FAMACHA test was also performed.

Two lambs were also culled and necropsy was performed to find any other information to help solve the case. Tissue specimens were collected from lungs, liver and muscles, placed into 10% formalin, stored and sent to OSU for analysis.

Ten ewes were selected based on poor lambing performance and clinical mastitis by physical examination. One sheep particularly, showed fever, reactive mammary lymphnodes, thinness, muscle weakness and mastitis in both mammary glands. Moreover, one teat had a fistula draining pus. A sample of purulent discharge was collected with a sterile swab, stored in a transport media (Stuarts Transport Medium, Thermo Scientific) and refrigerated until further processing at the Infectious Disease and Molecular Epidemiology Laboratory (IDMEL, OSU). The animal was euthanized as treatment was not feasible due to age.

Necropsy in lambs (n=3) revealed adhesions of right lung and chest wall; fibrin deposits on chest wall; apical lung lobe consolidated and firm; middle lung lobe consolidated and firm; pale musculature and carcass appearance; mesenteric lymph nodes prominent, color normal; liver pale; no evidence of nematodes (*Haemonchus*) in the abomasum; blood appeared pale; gastrointestinal contents normal. Biochemical tests revealed that lambs had mineral deficiency and, therefore, were more susceptible to infections and parasitic diseases resulting in unsatisfactory growth.

The swab sample collected from ewe was streaked onto Muller Hinton agar and incubated at 37 °C for 12h. Homogeneous colonies, circular, pinhead, convex with entire margins and light yellow were selected. These colonies were streaked onto mannitol salt agar (MSA, BD, Heidelberg,Germany) and oxacillin screen agar (Oxacillin Screen Agar, BD) and then, incubated at 37 °C for 24 hours. Growth was identified in both agars and colonies were then re-streaked onto Müller-Hinton agar plates and incubated for 24 h at 37 °C for further identification steps. Catalase positive colonies were tested by coagulase production by means of a commercial kit (Coagulase Plasma). For DNA extraction, we used QIAGEN DNeasy® Blood and Tissue kit for DNA extraction according to the manufacturer's protocol.

In order to identify MRSA, isolates were initially tested by PCR assays targeting the genes *nuc* and *mecA*. A 1 μL DNA template was added to a 24 μL master mix prepared by using a commercial kit (Illustra PuReTaq Ready-To-Go PCR beads, (GE Healthcare, UK) and primer sequences described in table 1 of supplementary material.

Sequencing analysis of isolates regarding seven housekeeping genes (arcC, aroE, glpF, gmk, pta, tpi, yqiL) was performed by a service provider (GeneWiz Inc, Boston, MA). Multiloccus Sequence Typing (MLST), findings were analyzed based on allelic profiles using MLST database (http://saureus.mlst.net/) and sequence types identification (ST).

Sample was *nuc* and *mecA* positive confirming genetically Methicillin resistant *S. aureus.* MLST revealed that tested MRSA isolate belonged to clonal lineage ST398.

Bacterial pathogens responsible for clinical and subclinical mastitis in small ruminants are well characterized. Sporadic cases of clinical mastitis most frequently are caused by *Staphylococcus aureus*, coagulase-negative *Staphylococcus spp*., *Arcanobacterium pyogenes, Corynebacterium, Pasteurella spp*., and *Pseudomonas spp*.(1)(2) Although clinical mastitis constitutes a small percentage of mastitis cases in small ruminants, usually less than 5%, it is usually the form of mastitis that producer is most aware of (1)(3). Clinical signs of mastitis include hard and swollen glands, enlarged supramammary lymph nodes, and possibly fever (4). Is large the impact of mastitis on lamb mortality in meat production herds(5). A low milk production due mastitis can lead to weakness of lambs, that inevitably will have a late growth and also be more susceptible to diseases. Probably, MRSA did not caused disease in lambs directly, but, find MRSA in a herd with a chronic history of mastitis make us to believe that they are related events. In contrast to bovine mastitis, little information is available in the field of molecular epidemiology of *S. aureus* recovered from ewe mastitis(6). MRSA is a frequent pathogen of humans and many animal species (7). In the past years, the

potential transmission of MRSA from animals to humans has gained special attention, especially in the case of the ST 398, a livestock associated MRSA (LA-MRSA). Recent epidemiological studies suggests have shown that this strain, particularly found in animal reservoirs (8–11), has also been associated with diseases in humans (12, 13). Therefore, this strain poses an increased threat to public health mainly as an occupational pathogen, potentially infecting farmers, handlers and even veterinarians.

To the best of our knowledge, this is the first MRSA report causing mastitis in sheep in USA. Mostly important, the identification of ST398 warrants further epidemiological investigations about the epidemiology this pathogen in animal production systems. Factors associated with the emergence of those agents are uncertain. Epidemiological investigations using molecular tools could provide key information about sources and transmission routes of MRSA as the basis to control the pathogen at animal production systems.

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References

1. **Pugh DG**. SHEEP AND GOAT MEDICINE 2nd Edition. Saunders, Elsevier Inc.
2. **Ciftci a.**, **Onuk EE**, **Findik a.**, **Yildirim T**, **Sogut MU**. 2009. Molecular Typing of *Staphylococcus Aureus* Strains from Ovine Mastitis by Pulsed-Field Gel Electrophoresis and Polymerase Chain Reaction Based on Coagulase and Protein A Gene Polymorphisms. J Vet Diagnostic Investig **21**:849–853.
3. **Chu C**, **Yu C**, **Lee Y**, **Su Y**. 2012. Genetically divergent methicillin-resistant *Staphylococcus aureus* and sec-dependent mastitis of dairy goats in Taiwan. BMC Vet Res **8**:39.
4. **Bergonier D**, **de Cremoux R**, **Rupp R**, **Lagriffoul G**, **Berthelot X**. 2003. Mastitis of dairy small ruminants. Vet Res **34**:689–716.
5. **Veríssimo CJ**, **Martins KB**, **Zafalon LF**. 2009. Mastitis in sheep : Lambs mortality. São Carlos -SP.
6. **Vautor E**, **Jay C**, **Chevalier N**, **Visomblin N**, **Vernet G**, **Pépin M**. 2005. Characterization of 26 isolates of *Staphylococcus aureus*, predominantly from dairy sheep, using four different techniques of molecular epidemiology. J Vet Diagn Invest **17**:363–368.
7. **Loncaric I**, **Brunthaler R**, **Spergser J**. 2013. Suspected goat-to-human transmission of methicillin-resistant *Staphylococcus aureus* sequence type 398. J Clin Microbiol **51**:1625–1626.
8. **Golding GR**, **Bryden L**, **Levett PN**, **McDonald RR**, **Wong A**, **Wylie J**, **Graham MR**, **Tyler S**, **van Domselaar G**, **Simor AE**, **Gravel D**, **Mulvey MR**. 2010. Livestock-associated methicillin-resistant *Staphylococcus*

aureus sequence type 398 in humans, Canada. Emerg Infect Dis **16**:587–594.

9. **Garcia-Graells C**, **van Cleef B a GL**, **Larsen J**, **Denis O**, **Skov R**, **Voss A**. 2013. Dynamic of Livestock-Associated Methicillin-Resistant *Staphylococcus aureus* CC398 in Pig Farm Households: A Pilot Study. PLoS One **8**:6–11.

10. **Harrison EM**, **Paterson GK**, **Holden MTG**, **Larsen J**, **Stegger M**, **Larsen AR**, **Petersen A**, **Skov RL**, **Christensen JM**, **Bak Zeuthen A**, **Heltberg O**, **Harris SR**, **Zadoks RN**, **Parkhill J**, **Peacock SJ**, **Holmes M a.** 2013. Whole genome sequencing identifies zoonotic transmission of MRSA isolates with the novel mecA homologue mecC. EMBO Mol Med **5**:509–515.

11. **Friese a.**, **Schulz J**, **Zimmermann K**, **Tenhagen B a.**, **Fetsch a.**, **Hartung J**, **Rösler U**. 2013. Occurrence of livestock-associated methicillin-resistant *Staphylococcus aureus* in turkey and broiler barns and contamination of air and soil surfaces in their vicinity. Appl Environ Microbiol **79**:2759–2766.

12. **Loncaric I**, **Brunthaler R**, **Spergser J**. 2013. Suspected Goat-to-Human Transmission of Methicillin-Resistant *Staphylococcus aureus* Sequence Type 398. J Clin Microbiol **51**:1625–1626.

13. **Hetem DJ**, **Bootsma MCJ**, **Troelstra A**, **Bonten MJM**. 2013. Transmissibility of livestock-associated methicillin-resistant *Staphylococcus aureus*. Emerg Infect Dis **19**:1797–1802.

CAPÍTULO 2

Occurrence of Methicillin Resistant *Staphylococcus aureus* (MRSA) in Dairy Goat Herds in Ohio, USA

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ABSTRACT

Methicillin resistant *Staphylococcus aureus* (MRSA) is major pathogen to humans and current knowledge suggest that animal production systems might play an important role on the emergence of MRSA strains. While there is no indication that MRSA is a foodborne pathogen, milk could be an important dissemination vehicle to humans, as staphylococci are the most important pathogens associated to subclinical mastitis in dairy animals. In view of the scarcity of information about the occurrence and characterization of MRSA in dairy goats, this study aimed to investigate the occurrence of Methicillin Resistant *S. aureus* (MRSA) associated with intramammary infections in goat herds in the State of Ohio, USA. A total of 120 milk samples and 120 teat-swab samples were collected from five farms. After conventional isolating and phenotypic characterization of the colonies, molecular characterization was also performed through PCR for *nuc* and *mecA* genes for MRSA confirmation and clonal complexes determined by MLST. Fifteen (6.2%) positive *S. aureus* samples were found in this study; nine from milk and five from teat skin samples. Four (2%) MRSA isolates were detected and belonged to clonal complexes ST133 and ST5. Three (1.25%) coagulase-negative isolates were shown to harbor the mecA gene. This study indicates that MRSA belonging to clonal complex 133 can be associated with intrammamary infections in goat herds in US and the identification of the MRSA ST5 is of special concern, since it's an important pathogen to humans.

Keywords: MRSA, goat herds, mastitis, MLST

INTRODUCTION

Mastitis is the most important and costly disease in dairy goat production. Animals can present physical, chemical, pathological and bacteriological changes in milk and glandular tissue (Cressier and Bissonnette, 2011). Subclinical mastitis is common in goats and is mainly caused by contagious bacteria. The most common agent of infectious mastitis in goats is *Staphylococcus aureus* (Nemeghaire et al., 2014) that can lead severe illnesses such as suppurative mastitis, arthritis, and urinary tract infection. *Staphylococcus aureus* has the ability to rapidly develop resistance to virtually any antibiotic drug used as therapeutic purposes and some lineages can also colonize and infect different hosts, including humans, causing a variety of infectious disorders (Pantosti, 2012).

As the most prevalent pathogen in both hospitals and communities, *S. aureus* can become methicillin (oxacillin) resistant *S. aureus* (MRSA/ORSA) by the introduction of an exogenous mobile staphylococcal chromosomal cassette, mec (SCCmec), encoding a low-affinity penicillin-binding protein 2a responsible for methicillin/oxacillin resistance (Chu et al., 2012). The potential for zoonotic transmission of *S. aureus* between livestock, companion animals and humans (Pantosti, 2012) has been exemplified by the emergence of MRSA ST398 (Neyra et al., 2014).

Thus, accurate and rapid detection and typing of *S. aureus* is crucial to control the infectious organisms among animal production systems. There is a scarcity of information about the occurrence of MRSA in the goat industry. This study aimed to detect Methicillin Resistant *S. aureus* (MRSA) and characterize the

clonal complexes of this pathogen involved in subclinical mastitis in goat herds in the State of Ohio, USA.

MATERIAL AND METHODS

Study design and Sampling

Farms were selected based on the College of Veterinary Medicine database at Ohio State University (OSU), Columbus, US. Farmers were invited to join the study by e-mail and then, based on agreement, 5 farms joined the experiment and visits were scheduled. Samplings were performed during the milking routine procedures in each farm and included teat skin swabs and milk. All the farms are located in a radius of 200 miles from Columbus and have Nubians, Toggenburgs and Saanen breeds as base of their herds. Dairy products are the primary activity in 3 of them and 4 properties participate in fairs and expositions commonly. The number of animals sampled and the milking routine of each farm is shown in the Table 1.

Table 1. Number of animals/farm and milking routine procedures.

Farms	Animals sampled	Type of Milking	Teat Dipping
Farm A	48	Parallel milking parlour	Not used
Farm B	22	Manual milking	Pre/post dipping
Farm C	15	Milking machine	Pre/post dipping
Farm D	20	Milking machine	Pre/post dipping
Farm E	15	Manual milking	Post dipping

Teat surface samplings were performed by rubbing a sterile swab onto teats and then, placing them into sterile tubes containing 5 ml Muller-Hinton broth with 6.5% NaCl. After cleaning the teats with 70% alcohol, milk was collected into 10ml sterile tube after discarding foremilk. A total of 120 animals were sampled and milk samples were kept under refrigeration conditions for transportation until processing at the Infectious Diseases and Molecular Epidemiology Laboratory (IDMEL-OSU).

Staphylococcus **isolating and Phenotypic Testing**

Samples were streaked onto Muller Hinton agar and incubated at 37 °C for 12h. Homogeneous colonies, circular, pinhead, convex with entire margins and light yellow were selected. These colonies were streaked onto mannitol salt agar (MSA, BD, Heidelberg,Germany) and oxacillin screen agar (Oxacillin Screen Agar, BD) and then, incubated at 37 °C for 24 hours (Figure 1). Growth was identified in both agars and colonies were then re-streaked onto Müller-Hinton agar plates and incubated for 24 h at 37 °C for further identification steps. Catalase positive colonies were tested by coagulase production by means of a commercial kit (BBL™ Coagulase Plasma, BD) (Figure 2) For DNA extraction, we used a commercial kit (QIAGEN DNeasy® Blood and Tissue kit) for DNA extraction according to the manufacturer's protocol.

Genotyping testing

In order to identify MRSA, isolates were tested by PCR assays targeting the genes *nuc* and *mecA*. A 1 *uL* DNA template was added to a 24 *uL* master mix prepared by using a commercial kit (Illustra PuReTaq Ready-To-Go PCR beads (GE Healthcare, UK) and primer sequences (Table 1).

Table 1. *Primers* and cycling conditions used in the identification of *Staphylococcus aureus*

			PCR Conditions		
Primers	**Primer Sequence (5'-3')**	**Amplicon Size**	**Denaturation**	**Annealing**	**Extension**
[1]nuc 1	TCAGCAAATGCATCACAAACAG	279 bp	94°C for 1 min	55°C for 0.5 min	72°C for 1.5 min
[1]nuc 2	CGTAAATGCACTTGCTTCAGG				
[2]mecA – F	GGGATCATAGCGTCATTATTC	533 bp	94°C for 3 min	50 °C for 1 min	72 °C for 1 min
[2]mecA – R	AACGATTGTGACACGATAGCC				

[1] CIFTCI *et al*., 2009; [2] DelVecchio et al., 1995

Electrophoresis of PCR products was performed on 1% agarose gel stained with ethidium bromide. DNA fragments were visualized in a UV transilluminator and photographed (Figure 3).

Clonal Complex Identification

Sequencing analysis of the isolates regarding seven housekeeping genes (*arcC, aroE, glpF, gmk, pta, tpi, yqiL*) was performed in a service provider (GeneWiz Inc, Boston, MA). Based on the sequence analysis, Multiloccus Sequence Typing (MLST) was determined by analyzing the allelic profiles using the MLST database (http://saureus.mlst .net/) and the identification of sequence types (ST).

RESULTS

Staphylococcal isolating results are shown in Table 2. Out of 240 collected samples, 53 (44,17%) isolates were recovered by MSA and 5 (4,17%) by ORSA considering skin samples. Considering milk samples, 12 (10%) of samples grew in MSA and 2 (1,7%) in ORSA.

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All samples which shown growth on MSA plates were tested for coagulase and catalase. Among that, 9 samples (13.8%) from milk and 6 (9.2%) samples from skin were confirmed coagulase positive. Those fifteen (6.2%) positive *S. aureus* samples were further confirmed as *S. aureus* by PCR and four (2%) were confirmed MRSA by testing mecA positive. were detected. Three (1.25%) coagulase negative staphylococci isolates carrying the *mecA* gene were also detected.

Among the 4 MRSA positive samples, 3 (75%) were recovered from farm A and 1 (25%) from the farm E.

Table 2. Frequencies of *Staphylococcus aureus*, Coagulase Negative *Staphylococcus spp.* (CNS), Methicillin Resistant *Staphylococcus aureus* (MRSA) and Methicillin Resistant Coagulase negative *Staphylococcus spp.* (MR-CNS) in milk and skin samples.

	S. aureus	**CoNS**	**MRSA**	**MR-CNS**
Milk (120)	**9** (7,5%)	**3** (2,5%)	**2** (1,7%)	**0**
Skin (120)	**6** (5%)	**47** (39,1%)	**2** (1,7%)	**3** (2,5%)
TOTAL 240 (100%)	**15** (6,2%)	**50** (20,83%)	**4** (1,7%)	**3** (1,25%)

MLST was performed to determine the clonal complex (CC) of the 4 MRSA isolates. Two isolates were defined as belonging to CC133, both from milk samples and the other two, to CC5.

DISCUSSION

The frequency of *S. aureus* detected in this study was lower than found in Europe (Stastkova et al., 2009), Asia (Kwan et al., 2005) and South America (Peixoto et al., 2010). *Staphylococcus aureus* has been considered the major mastitis causing agent in sheep and goats (Bergonier et al., 2003). However, an increasing number of CoNS has been reported as the causative agent of mastitis in small ruminants. Among goats, 25 to 93% of the mastitis cases has been associated to CoNS and this must be taken into consideration from the point of view of antibiotic selection for mastitis treatment, especially if the possibility exists of the resistance transfer in or between microbial species (Vyletelova et al., 2011).

MRSA frequently colonizes animals, especially livestock, and that has been a reason for concern (Golding et al., 2010). Since 2005, MRSA belonging to ST398 was recognized as a colonizer of pigs and human subjects professionally exposed to pig farming (Hetem et al., 2013). These strains have acquired antibiotic resistance due to selective pressures in the pig farming industry and have the capacity to cause severe zoonotic infections of humans in contact with pigs (Smith et al., 2013).

The MRSA strains identified in this study belonged to CC133. This clonal complex comprises 17 STs that were confirmed to be associated with intramammary infections of cows, sheep and goats, along with six STs of unknown origin, constituting the largest animal-associated CC within *S. aureus* and comprising strains from several different geographical locations (Norway, France, UK, Portugal, Brazil, Ireland, Sweden, USA, Italy, Iceland and Austria) (Smyth et al., 2009).

ST5 is a human-associated type predominant in HA-MRSA (Jackson et al., 2013). ST5 has been previously found in retail pork and beef in the United States, as has a poultry- adapted clone that was reported to have originated in humans (Waters et al., 2011) and it´s consider one of major international Epidemic MRSA (EMRSA) (Jamrozy et al., 2012)(Enright et al., 2002). ST5 has already been recovered from bovine mastitis in Japan, and this finding is uncommon among bovine isolates in the world, what could indicate the potential emergence of MRSA from dairy environment (Hata et al., 2010).

The farm A, where 3 (75%) MRSA were detected, showed poor sanitary practices that could lead to animal health problems such as mastitis. For instance, neither pre nor post-dipping, which are highly recommended in dairy goats management (Hedrich et al., 2008), are used in the milking routine in this farm. It has been demonstrated that hygiene measures in dairy goat production are effective in reducing *S. aureus*-associated intrammamary infections and thus improve milk quality (Garcia-Graells et al., 2013) (Hedrich et al., 2008). It's worth note that milk pasteurization was not used in that farm, which emphasize the importance of milk and dairy products as vehicles of zoonotic bacteria to consumers.

In summary, this study indicates that MRSA belonging to clonal complex 133 can be associated with intrammamary infections in goat herds in US and the identification of the MRSA ST5 is of special concern, since it's an important pathogen to humans. Lastly, although the limited number of farms sampled, the fact that most MRSA isolates (75%) were originated from the farm showing less hygiene practices among indicate the necessity to understand potential risk factors for the occurrence of MRSA in goat dairy farms. Moreover, further studies are required to inestigate the role of MRSA as a cause of contagious subclinical intramammary infection in the goat species.

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REFERENCES

Bergonier, D., de Crémoux, R., Rupp, R., Lagriffoul, G., Berthelot, X., 2003. Mastitis of dairy small ruminants. Vet. Res. 34, 689–716. doi:10.1051/vetres:2003030

Chu, C., Yu, C., Lee, Y., Su, Y., 2012. Genetically divergent methicillin-resistant *Staphylococcus aureus* and sec-dependent mastitis of dairy goats in Taiwan. BMC Vet. Res. 8, 39. doi:10.1186/1746-6148-8-39

Cressier, B., Bissonnette, N., 2011. Assessment of an extraction protocol to detect the major mastitis-causing pathogens in bovine milk. J. Dairy Sci. 94, 2171–2184. doi:10.3168/jds.2010-3669

DelVecchio, V.G., Petroziello, J.M., Gress, M.J., McCleskey, F.K., Melcher, G.P., Crouch, H.K., Lupski, J.R., 1995. Molecular genotyping of methicillin-resistant *Staphylococcus aureus* via fluorophore-enhanced repetitive-sequence PCR. J. Clin. Microbiol. 33, 2141–2144.

Enright, M.C., Robinson, D.A., Randle, G., Feil, E.J., Grundmann, H., Spratt, B.G., 2002. The evolutionary history of methicillin-resistant *Staphylococcus aureus* (MRSA) 99.

Garcia-Graells, C., van Cleef, B. a G.L., Larsen, J., Denis, O., Skov, R., Voss, A., 2013. Dynamic of Livestock-Associated Methicillin-Resistant *Staphylococcus aureus* CC398 in Pig Farm Households: A Pilot Study. PLoS One 8, 6–11. doi:10.1371/journal.pone.0065512

Golding, G.R., Bryden, L., Levett, P.N., McDonald, R.R., Wong, A., Wylie, J., Graham, M.R., Tyler, S., van Domselaar, G., Simor, A.E., Gravel, D., Mulvey, M.R., 2010. Livestock-associated methicillin-resistant *Staphylococcus aureus* sequence type 398 in humans, Canada. Emerg. Infect. Dis. 16, 587–594. doi:10.3201/eid1604.091435

Hata, E., Katsuda, K., Kobayashi, H., Uchida, I., Tanaka, K., Eguchi, M., 2010. Genetic variation among *Staphylococcus aureus* strains from bovine milk and their relevance to methicillin-resistant isolates from humans. J. Clin. Microbiol. 48, 2130–2139. doi:10.1128/JCM.01940-09

Hedrich, C., Duelmer, C., Considine, D., 2008. Dairy Goat Management Best Management Practices.

Hetem, D.J., Bootsma, M.C.J., Troelstra, A., Bonten, M.J.M., 2013. Transmissibility of livestock-associated methicillin-resistant *Staphylococcus aureus*. Emerg. Infect. Dis. 19, 1797–1802. doi:10.3201/eid1911.121085

Jackson, C.R., Davis, J. a., Barrett, J.B., 2013. Prevalence and characterization of methicillin-resistant *Staphylococcus aureus* isolates from retail meat and humans in Georgia. J. Clin. Microbiol. 51, 1199–1207. doi:10.1128/JCM.03166-12

Jamrozy, D.M., Fielder, M.D., Butaye, P., Coldham, N.G., 2012. Comparative genotypic and phenotypic characterisation of methicillin-resistant *Staphylococcus aureus*

ST398 isolated from animals and humans. PLoS One 7. doi:10.1371/journal.pone.0040458

Kwan, S.K., Lee, J.Y., Ji, Y.S., Won, S.O., Kyong, R.P., Nam, Y.L., Song, J.H., 2005. Distribution of major genotypes among methicillin-resistant *Staphylococcus aureus* clones in Asian countries. J. Clin. Microbiol. 43, 421–426. doi:10.1128/JCM.43.1.421-426.2005

Nemeghaire, S., Argudín, M.A., Haesebrouck, F., Butaye, P., 2014. Epidemiology and molecular characterization of methicillin-resistant *Staphylococcus aureus* nasal carriage isolates from bovines. BMC Vet. Res. 10, 153. doi:10.1186/1746-6148-10-153

Neyra, R.C., Frisancho, J.A., Rinsky, J.L., Resnick, C., Carroll, K.C., Rule, A.M., Ross, T., You, Y., Price, L.B., Silbergeld, E.K., 2014. Multidrug-Resistant and Methicillin-Resistant. Environ. Health Perspect. 122, 471–477.

Pantosti, A., 2012. Methicillin-resistant *Staphylococcus aureus* associated with animals and its relevance to human health. Front. Microbiol. 3, 1–12. doi:10.3389/fmicb.2012.00127

Peixoto, R. de M., Mota, R.A., Costa, M.M. da, 2010. Small ruminant mastitis in Brazil. Pesqui. Veterinária Bras. 30, 754–762. doi:10.1590/S0100-736X2010000900008

Smith, T.C., Gebreyes, W. a., Abley, M.J., Harper, A.L., Forshey, B.M., Male, M.J., Martin, H.W., Molla, B.Z., Sreevatsan, S., Thakur, S., Thiruvengadam, M., Davies, P.R., 2013. Methicillin-Resistant *Staphylococcus aureus* in Pigs and Farm Workers on Conventional and Antibiotic-Free Swine Farms in the USA. PLoS One 8, 1–5. doi:10.1371/journal.pone.0063704

Smyth, D.S., Feil, E.J., Meaney, W.J., Hartigan, P.J., Tollersrud, T., Fitzgerald, J.R., Enright, M.C., Smyth, C.J., 2009. Molecular genetic typing reveals further insights into the diversity of animal-associated *Staphylococcus aureus*. J. Med. Microbiol. 58, 1343–1353. doi:10.1099/jmm.0.009837-0

Stastkova, Z., Karpiskova, S., Karpiskova, R., 2009. Occurrence of methicillin-resistant strains of *Staphylococcus aureus* at a goat breeding farm. Vet. Med. (Praha). 54, 419–426.

Vyletelova, M., Vlkova, H., Manga, I., 2011. Occurrence and Characteristics of Methicillin Resistant Staphylococcus aureus and Methicillin Resistant Coagulase-negative *Staphylococc*i in Raw Milk Manufacturing. Czech J. Food Sci. 29, S11–S16.

Waters, A.E., Contente-Cuomo, T., Buchhagen, J., Liu, C.M., Watson, L., Pearce, K., Foster, J.T., Bowers, J., Driebe, E.M., Engelthaler, D.M., Keim, P.S., Price, L.B., 2011. Multidrug-resistant *Staphylococcus aureus* in US meat and poultry. Clin. Infect. Dis. 52, 1227–1230. doi:10.1093/cid/cir181

CONSIDERAÇÕES FINAIS

A mastite é uma doença que afeta todos os mamíferos de produção, entretanto, o maior foco está nos rebanhos leiteiros. O caso em questão, o proprietário procurou o serviço veterinário da Universidade pra resolver um problema que ele acreditava ser primariamente algo nos cordeiros mas que, na verdade, tratava-se de uma deficiência nutricional dos animais jovens pois as matrizes que apresentavam mastite não produziam leite em quantidade e qualidade suficientes para garantir uma boa alimentação na fase crítica de desenvolvimento dos animais, o que mostra que a mastite também é um problema sério em rebanhos produtores de carne.

Devemos atentar para os animais positivos para MRSA já que estes podem ser considerados reservatórios e disseminadores do patógeno para outros animais, seres humanos e ao ambiente. Além disso,as enfermidades causadas por MRSA são consideradas sérias para saúde pública, o que remete a importância de sempre manter o monitoramento das cepas resistêntes nas mais diversas fontes incluindo os animais de produção através de técnicas de epidemiologia molecular determinando a origem destas, ajudando no desenvolvimento de medidas profiláticas adequedas.

Este estudo é o primeiro a relatar a infecção de uma ovelha pela cepa MRSA CC398 em um rebanho ovino norte-americano. Isso é importante uma vez que, sem esse conhecimento, é impossível de controlar de forma eficaz este organismo responsável por grande parte das infecções intramamárias clínicas sendo também um patógeno humano importante e motivo de grandes preocupações em saúde pública.

REFERÊNCIAS

AUTHORITY, E. F. S. Analysis of the baseline survey on the prevalence of methicillin-resistant *Staphylococcus aureus* (MRSA) in holdings with breeding pigs, in the EU, 2008. Part A: MRSA prevalence estimates. **EFSA Journal**, v. 7, n. 11, p. 1376, 2009.

BERGONIER, D.; DE CREMOUX, R.; RUPP, R.; LAGRIFFOUL, G.; BERTHELOT, X. Mastitis of dairy small ruminants. **Veterinary Research**, v. 34, n. 5, p. 689–716, 2003. Disponível em: <http://www.edpsciences.org/10.1051/vetres:2003030>. Acesso em: 15/5/2014.

BERGONIER, D.; DE CRÉMOUX, R.; RUPP, R.; LAGRIFFOUL, G.; BERTHELOT, X. Mastitis of dairy small ruminants. **Veterinary research**, v. 34, n. 5, p. 689–716, 2003. Disponível em: <http://www.ncbi.nlm.nih.gov/pubmed/14556701>. .

CHU, C.; YU, C.; LEE, Y.; SU, Y. Genetically divergent methicillin-resistant *Staphylococcus aureus* and sec-dependent mastitis of dairy goats in Taiwan. **BMC veterinary research**, v. 8, p. 39, 2012. Disponível em: <http://www.ncbi.nlm.nih.gov/pubmed/22455622>. .

CIFTCI, A.; ONUK, E. E.; FINDIK, A.; YILDIRIM, T.; SOGUT, M. U. Molecular Typing of *Staphylococcus Aureus* Strains from Ovine Mastitis by Pulsed-Field Gel Electrophoresis and Polymerase Chain Reaction Based on Coagulase and Protein A Gene Polymorphisms. **Journal of Veterinary Diagnostic Investigation**, v. 21, p. 849–853, 2009.

CONCORDE. **Control of community-acquired MRSA : rationale and development of counteractions**. 2013.

CRESSIER, B.; BISSONNETTE, N. Assessment of an extraction protocol to detect the major mastitis-causing pathogens in bovine milk. **Journal of dairy science**, v. 94, n. 5, p. 2171–2184, 2011. Disponível em: <http://www.ncbi.nlm.nih.gov/pubmed/21524507>. .

DELVECCHIO, V. G.; PETROZIELLO, J. M.; GRESS, M. J.; et al. Molecular genotyping of methicillin-resistant *Staphylococcus aureus* via fluorophore-enhanced repetitive-sequence PCR. **Journal of Clinical Microbiology**, v. 33, n. 8, p. 2141–2144, 1995.

ELLAYNE SOUZA CERQUEIRA, R. C. DE C. A. *Staphylococcus aureus* resistente à meticilina (MRSA) em alimentos de origem animal : uma revisão sistemática Methicillin-resistant *Staphylococcus aureus* (MRSA) in foods of animal origin : A systematic review. **Instituto Adolfo Lutz**, v. 72, n. 4, p. 268–281, 2013.

ENRIGHT, M. C.; ROBINSON, D. A.; RANDLE, G.; et al. The evolutionary history of methicillin-resistant *Staphylococcus aureus* (MRSA). , v. 99, n. 11, 2002.

FRIESE, A.; SCHULZ, J.; ZIMMERMANN, K.; et al. Occurrence of livestock-associated methicillin-resistant *Staphylococcus aureus* in turkey and broiler barns and contamination of air and soil surfaces in their vicinity. **Applied and Environmental Microbiology**, v. 79, n. 8, p. 2759–2766, 2013.

GARCIA-GRAELLS, C.; VAN CLEEF, B. A G. L.; LARSEN, J.; et al. Dynamic of Livestock-Associated Methicillin-Resistant *Staphylococcus aureus* CC398 in Pig Farm Households: A Pilot Study. **PLoS ONE**, v. 8, n. 5, p. 6–11, 2013.

GOLDING, G. R.; BRYDEN, L.; LEVETT, P. N.; et al. Livestock-associated methicillin-resistant *Staphylococcus aureus* sequence type 398 in humans, Canada. **Emerging Infectious Diseases**, v. 16, n. 4, p. 587–594, 2010.

HARRISON, E. M.; PATERSON, G. K.; HOLDEN, M. T. G.; et al. Whole genome sequencing identifies zoonotic transmission of MRSA isolates with the novel *mecA* homologue *mecC*. **EMBO Molecular Medicine**, v. 5, p. 509–515, 2013.

HATA, E.; KATSUDA, K.; KOBAYASHI, H.; et al. Genetic variation among *Staphylococcus aureus* strains from bovine milk and their relevance to methicillin-resistant isolates from humans. **Journal of Clinical Microbiology**, v. 48, n. 6, p. 2130–2139, 2010.

HEDRICH, C.; DUELMER, C.; CONSIDINE, D. **Dairy Goat Management Best Management Practices**. 2008.

HETEM, D. J.; BOOTSMA, M. C. J.; TROELSTRA, A.; BONTEN, M. J. M. Transmissibility of livestock-associated methicillin-resistant *Staphylococcus aureus*. **Emerging Infectious Diseases**, v. 19, n. 11, p. 1797–1802, 2013.

JACKSON, C. R.; DAVIS, J. A.; BARRETT, J. B. Prevalence and characterization of methicillin-resistant *Staphylococcus aureus* isolates from retail meat and humans in Georgia. **Journal of Clinical Microbiology**, v. 51, n. 4, p. 1199–1207, 2013.

JAMROZY, D. M.; FIELDER, M. D.; BUTAYE, P.; COLDHAM, N. G. Comparative genotypic and phenotypic characterisation of methicillin-resistant *Staphylococcus aureus* ST398 isolated from animals and humans. **PLoS ONE**, v. 7, n. 7, 2012.

KWAN, S. K.; LEE, J. Y.; JI, Y. S.; et al. Distribution of major genotypes among methicillin-resistant *Staphylococcus aureus* clones in Asian countries. **Journal of Clinical Microbiology**, v. 43, n. 1, p. 421–426, 2005.

LONCARIC, I.; BRUNTHALER, R.; SPERGSER, J. Suspected Goat-to-Human Transmission of Methicillin-Resistant *Staphylococcus aureus* Sequence Type 398. **Journal of Clinical Microbiology**, v. 51, n. 5, p. 1625–1626, 2013. Disponível em: <http://jcm.asm.org/cgi/doi/10.1128/JCM.03052-12>. Acesso em: 15/5/2014.

NEMEGHAIRE, S.; ARGUDÍN, M. A.; HAESEBROUCK, F.; BUTAYE, P. Epidemiology and molecular characterization of methicillin-resistant *Staphylococcus aureus* nasal carriage isolates from bovines. **BMC veterinary research**, v. 10, n. 1, p. 153, 2014. Disponível em: <http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=4103977&tool=pmcentrez&rendertype=abstract>. .

NEYRA, R. C.; FRISANCHO, J. A.; RINSKY, J. L.; et al. Multidrug-Resistant and Methicillin-Resistant. **Environmental Health Perspectives**, v. 122, n. 5, p. 471–477, 2014.

NIENHOFF, U.; KADLEC, K.; CHABERNY, I. F.; et al. Transmission of methicillin-resistant *Staphylococcus aureus* strains between humans and dogs: Two case reports. **Journal of Antimicrobial Chemotherapy**, v. 64, n. 3, p. 660–662, 2009.

PANTOSTI, A. Methicillin-resistant *Staphylococcus aureus* associated with animals and its relevance to human health. **Frontiers in Microbiology**, v. 3, n. April, p. 1–12, 2012.

PEIXOTO, R. DE M.; MOTA, R. A.; COSTA, M. M. DA. Small ruminant mastitis in Brazil. **Pesquisa Veterinária Brasileira**, v. 30, n. 9, p. 754–762, 2010. Disponível em: <http://www.scielo.br/scielo.php?script=sci_abstract&pid=S0100-736X2010000900008&lng=en&nrm=iso&tlng=pt>. Acesso em: 15/5/2014.

PISANU, S.; CUBEDDU, T.; PAGNOZZI, D.; et al. Neutrophil extracellular traps in sheep mastitis. **Veterinary Research**, v. 46, n. 1, p. 59, 2015. Veterinary Research. Disponível em: <http://www.veterinaryresearch.org/content/46/1/59>. .

PUGH, D. G. **SHEEP AND GOAT MEDICINE**. 2nd Edition ed.Saunders, Elsevier Inc, .

SANTANA, E. H. W. DE; BELOTI, V.; ARAGON-ALEGRO, L. C.; MENDONÇA, M. B. O. C. Estafilococos em Alimentos. **Arquivos do Instituto Biológico**, v. 77, n. 3, p. 545–554, 2010.

SMITH, T. C.; GEBREYES, W. A.; ABLEY, M. J.; et al. Methicillin-Resistant *Staphylococcus aureus* in Pigs and Farm Workers on Conventional and Antibiotic-Free Swine Farms in the USA. **PLoS ONE**, v. 8, n. 5, p. 1–5, 2013.

SMYTH, D. S.; FEIL, E. J.; MEANEY, W. J.; et al. Molecular genetic typing reveals further insights into the diversity of animal-associated *Staphylococcus aureus*. **Journal of Medical Microbiology**, v. 58, n. 10, p. 1343–1353, 2009.

STASTKOVA, Z.; KARPISKOVA, S.; KARPISKOVA, R. Occurrence of methicillin-resistant strains of *Staphylococcus aureus* at a goat breeding farm. **Veterinarni Medicina**, v. 54, n. 9, p. 419–426, 2009.

VAUTOR, E.; JAY, C.; CHEVALIER, N.; et al. Characterization of 26 isolates of *Staphylococcus aureus*, predominantly from dairy sheep, using four different techniques of molecular epidemiology. **Journal of veterinary diagnostic investigation : official publication of the American Association of Veterinary Laboratory Diagnosticians, Inc**, v. 17, p. 363–368, 2005.

VELASCO, V.; SHERWOOD, J. S.; ROJAS-GARCÍA, P. P.; LOGUE, C. M. Multiplex Real-Time PCR for Detection of *Staphylococcus aureus*, *mecA* and Panton-Valentine Leukocidin (PVL) Genes from Selective Enrichments from Animals and Retail Meat. **PLoS ONE**, v. 9, n. 5, p. e97617, 2014. Disponível em: <http://dx.plos.org/10.1371/journal.pone.0097617>. .

VYLETELOVA, M.; VLKOVA, H.; MANGA, I. Occurrence and Characteristics of Methicillin Resistant *Staphylococcus aureus* and Methicillin Resistant Coagulase-negative *Staphylococci* in Raw Milk Manufacturing. **Czech Journal of Food Sciences**, v. 29, p. S11–S16, 2011. Disponível em: <ISI:000299721700002>. .

WATERS, A. E.; CONTENTE-CUOMO, T.; BUCHHAGEN, J.; et al. Multidrug-resistant *Staphylococcus aureus* in US meat and poultry. **Clinical Infectious Diseases**, v. 52, n. 10, p. 1227–1230, 2011.

ZASTEMPOWSKA, E.; LASSA, H. Genotypic characterization and evaluation of an antibiotic resistance of *Trueperella pyogenes* (*Arcanobacterium pyogenes*) isolated from milk of dairy cows with clinical mastitis. **Veterinary Microbiology**, v. 161, n. 1–2, p. 153–158, 2012. Disponível em: <http://www.sciencedirect.com/science/article/pii/S0378113512004038>. Acesso em: 9/5/2014.

APÊNDICES

Apêndice A. Supplementary Material

Journal of Clinical Microbiology

Livestock-Associated MRSA ST398 in a sheep herd - USA.

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Department of Animal Sciences, College of ACES, University of Illinois at Urbana-Champaign (UIUC), IL – USA[4]

(Supplementary Material)

DNA extraction protocol

1. Harvest bacteria in 1000-1500 µl Molecular Grade Water in a sterile 1.5 mL microcentrifuge tube.
2. Centrifuge for 10 min at 14000 rpm
3. Discard supernatant
4. Resuspend pellet in 180 µl prepared enzymatic lysis buffer (see above) and vortex the samples.
5. Incubate for at least 30 min at 37°C
6. Remove samples from heating block and vortex to mix.
7. Add 25 µl proteinase K and 200 µl Buffer AL and mix by vortexing.
8. Incubate at 56°C for 30 min.
9. Remove samples from heating block and vortex to mix
10. Add 200 µl ethanol (96–100%) to the sample, and mix thoroughly by vortexing.
11. Pipette the mixture (including any precipitate) into the appropriate labeled spin column
12. Centrifuge at 8,000-10,000 rpm for 1 min. Discard collection tube.
13. Place the spin column in a new collection tube.
14. Add 500 µl Buffer AW1.
15. Centrifuge for 1 min at 8,000-10,000 rpm. Discard collection tube.
16. Place the spin column in a new collection tube.
17. Add 500 µl Buffer AW2.
18. Centrifuge for 3 min at 14,000 rpm. Discard collection tube.
19. Place the spin column in a 1.5 ml microcentrifuge tube.
20. Pipet 100 µl Molecular Grade Water (if sample will be used for sequencing) or 100 µl Buffer AE (NOT if sample is to be used for sequencing) into spin column.
21. Incubate at room temperature for 1 min.
22. Centrifuge for 1 min at 8000 rpm.
23. Store elute at 4°C

Supplementary Table 1. *Primers* and cycling conditions used in the identification of *Staphylococcus aureus*

			PCR Conditions		
Primers	**Primer Sequence (5'-3')**	**Amplicon Size**	**Denaturation**	**Annealing**	**Extension**
[1]nuc 1	TCAGCAAATGCATCACAAACAG	279 bp	94°C for 1 min	55°C for 0.5 min	72°C for 1.5 min
[1]nuc 2	CGTAAATGCACTTGCTTCAGG				
[2]mecA – F	GGGATCATAGCGTCATTATTC	533 bp	94°C for 3 min	50 °C for 1 min	72 °C for 1 min
[2]mecA – R	AACGATTGTGACACGATAGCC				

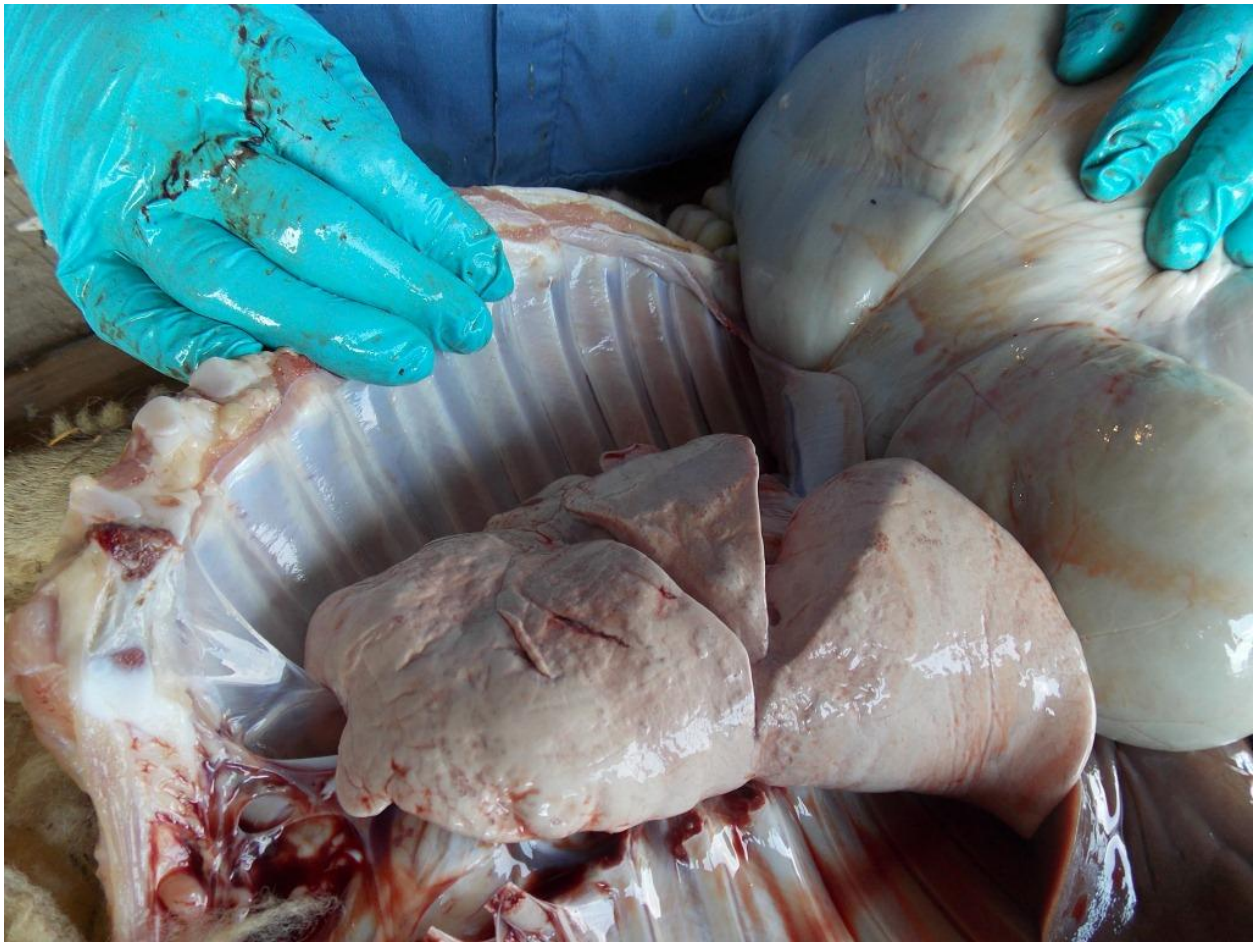
[1] CIFTCI *et al*., 2009; [2] Del Vechio et al, 1995

Supplementary table 2. Lambs clinical monitoring spreadsheet

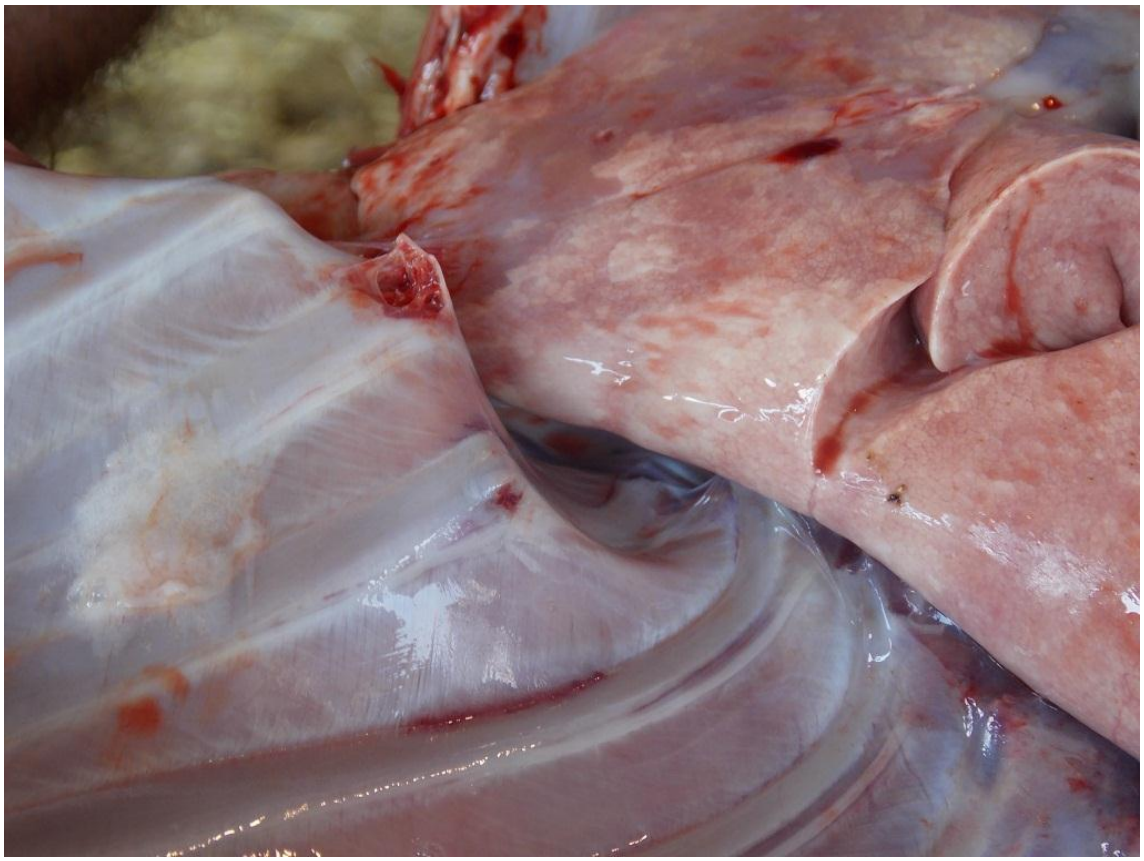
	03-07-12			**18-07-12**		
#ID	**weight**	**FAMACHA**	**FEC***	**weight**	**FAMACHA**	**FEC***
2126	41	2	2000	41	1	100
2093	43	1	750	44	1	0
2046	58	2	450	59	1	350
2021	37	2	2100	39	2	100
2074	37	3 or 2	2200	39	2	0
2120	41	2	550	40	2	100
2121	37	2	1700	39	2	0
2130	36	2	350	39	2	0
2044	63	2	650	64	1	0
2087	44	2	700	44	2	200
2023	43	2	1950	46	1	1200
2123	54	1	600	53	1	0
2143	44	2	2300	46	1	0
2144	40	2	1500	45	1	0
2147	40	2	0	43	1	0
2037	43	2 or 3	1250	44	2	0
2118	35	2	1800	36	2	50
2067	41	3	600	42	2	400
2036	53	2	1550	50	2	300
2131	43	3	700	44	2	0
2017	38	2	772	41	2	0
2022	49	3	900	51	2	50
2027	45	2	1500	51	1	0

2012	52	2	1100	53	2	0
2055	36	2	350	38	2	50

Supplementary figure 1.Necropsy: apical lung lobe consolidated and firm; middle lung lobe consolidated and firm.



Supplementary figure 2. Necropsy: adhesions of right lung and chest wall; fibrin deposits on chest wall.



Apêndice B. Supplementary Material

Occurrence of Methicillin Resistant *Staphylococcus aureus* (MRSA) in Dairy Goat Herds in Ohio, USA

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Veterinary Public Health and Biotechnology Global Consortium (VPH-Biotec), The Ohio State University, USA[3]

(Supplementary Material)

DNA extraction protocol

1. Harvest bacteria in 1000-1500 µl Molecular Grade Water in a sterile 1.5 mL microcentrifuge tube.
2. Centrifuge for 10 min at 14000 rpm
3. Discard supernatant
4. Resuspend pellet in 180 µl prepared enzymatic lysis buffer (see above) and vortex the samples.
5. Incubate for at least 30 min at 37°C
6. Remove samples from heating block and vortex to mix.
7. Add 25 µl proteinase K and 200 µl Buffer AL and mix by vortexing.
8. Incubate at 56°C for 30 min.
9. Remove samples from heating block and vortex to mix
10. Add 200 µl ethanol (96–100%) to the sample, and mix thoroughly by vortexing.
11. Pipette the mixture (including any precipitate) into the appropriate labeled spin column
12. Centrifuge at 8,000-10,000 rpm for 1 min. Discard collection tube.
13. Place the spin column in a new collection tube.
14. Add 500 µl Buffer AW1.
15. Centrifuge for 1 min at 8,000-10,000 rpm. Discard collection tube.
16. Place the spin column in a new collection tube.
17. Add 500 µl Buffer AW2.
18. Centrifuge for 3 min at 14,000 rpm. Discard collection tube.
19. Place the spin column in a 1.5 ml microcentrifuge tube.
20. Pipet 100 µl Molecular Grade Water (if sample will be used for sequencing) or 100 µl Buffer AE (NOT if sample is to be used for sequencing) into spin column.
21. Incubate at room temperature for 1 min.
22. Centrifuge for 1 min at 8000 rpm.
23. Store elute at 4°C

Suplementary Table 1. Phenotypic and genotypic characterization of isolates

#Sample[1]	Specie	Type of Sample	MSA	ORSA	Coagulase	Catalase	*nuc* Gene*	*mecA* Gene*
1A	Goat	Milk	Positive	Positive	Positive	Positive	1	1
19A	Goat	Milk	Positive	Negative	Positive	Positive	1	0
24A	Goat	Milk	Positive	Negative	Positive	Positive	1	0
25A	Goat	Milk	Positive	Negative	Positive	Positive	1	0
30A	Goat	Milk	Positive	Negative	Positive	Positive	1	0
5B	Goat	Milk	Positive	Negative	Negative	Positive	0	0
9B	Goat	Milk	Positive	Negative	Negative	Positive	0	0
4C	Goat	Milk	Positive	Negative	Positive	Positive	1	0
10D	Goat	Milk	Positive	Negative	Positive	Positive	1	0
2E	Goat	Milk	Positive	Negative	Positive	Positive	1	0
6E	Goat	Milk	Positive	Negative	Negative	Positive	0	0
8E	Goat	Milk	Positive	Positive	Positive	Positive	1	1
#Sample	Specie	Type of Sample	MSA	ORSA	Coagulase	Catalase	Nuc Gen	MecA Gen
1A	Goat	Teat Swab	Positive	Positive	Positive	Positive	1	1
2A	Goat	Teat Swab	Positive	Negative	Negative	Positive	0	0
3A	Goat	Teat Swab	Positive	Negative	Negative	Positive	0	0
4A	Goat	Teat Swab	Positive	Negative	Negative	Positive	0	0
5A	Goat	Teat Swab	Positive	Negative	Negative	Positive	0	0
6A	Goat	Teat Swab	Positive	Negative	Negative	Positive	0	0
7A	Goat	Teat Swab	Positive	Negative	Negative	Positive	0	0
8A	Goat	Teat Swab	Positive	Positive	Negative	Positive	0	1
9A	Goat	Teat Swab	Positive	Negative	Negative	Positive	0	0
10A	Goat	Teat Swab	Positive	Negative	Negative	Positive	0	0
11A	Goat	Teat Swab	Positive	Negative	Negative	Positive	0	0
12A	Goat	Teat Swab	Positive	Negative	Negative	Positive	0	0
13A	Goat	Teat Swab	Positive	Negative	Negative	Positive	0	0
14A	Goat	Teat Swab	Positive	Negative	Negative	Positive	0	0
19A	Goat	Teat Swab	Positive	Positive	Positive	Positive	1	0
16A	Goat	Teat Swab	Positive	Negative	Negative	Positive	0	0
24A	Goat	Teat Swab	Positive	Positive	Positive	Positive	1	0
25A	Goat	Teat Swab	Positive	Positive	Positive	Positive	1	0
1B	Goat	Teat Swab	Positive	Negative	Negative	Positive	0	0
2B	Goat	Teat Swab	Positive	Positive	Negative	Positive	0	1
3B	Goat	Teat Swab	Positive	Negative	Negative	Positive	0	0
4B	Goat	Teat Swab	Positive	Negative	Negative	Positive	0	0
5B	Goat	Teat Swab	Positive	Negative	Negative	Positive	0	0
8B	Goat	Teat Swab	Positive	Negative	Negative	Positive	0	0
10B	Goat	Teat Swab	Positive	Negative	Negative	Positive	0	0
12B	Goat	Teat Swab	Positive	Negative	Negative	Positive	0	0
13B	Goat	Teat Swab	Positive	Negative	Negative	Positive	0	0
14B	Goat	Teat Swab	Positive	Negative	Negative	Positive	0	0
15B	Goat	Teat Swab	Positive	Negative	Negative	Positive	0	0

1C	Goat	Teat Swab	Positive	Negative	Negative	Positive	0	0
2C	Goat	Teat Swab	Positive	Negative	Negative	Positive	0	0
3C	Goat	Teat Swab	Positive	Negative	Negative	Positive	0	0
4C	Goat	Teat Swab	Positive	Negative	Negative	Positive	0	0
7C	Goat	Teat Swab	Positive	Negative	Negative	Positive	0	0
11C	Goat	Teat Swab	Positive	Negative	Negative	Positive	0	0
12C	Goat	Teat Swab	Positive	Negative	Negative	Positive	0	0
13C	Goat	Teat Swab	Positive	Negative	Negative	Positive	0	0
15C	Goat	Teat Swab	Positive	Negative	Negative	Positive	0	0
1D	Goat	Teat Swab	Positive	Negative	Negative	Positive	0	0
2D	Goat	Teat Swab	Positive	Negative	Negative	Positive	0	0
4D	Goat	Teat Swab	Positive	Negative	Negative	Positive	0	0
5D	Goat	Teat Swab	Positive	Negative	Negative	Positive	0	0
6D	Goat	Teat Swab	Positive	Negative	Negative	Positive	0	0
7D	Goat	Teat Swab	Positive	Negative	Negative	Positive	0	0
8D	Goat	Teat Swab	Positive	Negative	Negative	Positive	0	0
9D	Goat	Teat Swab	Positive	Negative	Negative	Positive	0	0
10D	Goat	Teat Swab	Positive	Positive	Positive	Positive	1	0
3E	Goat	Teat Swab	Positive	Negative	Negative	Positive	0	0
4E	Goat	Teat Swab	Positive	Positive	Negative	Positive	0	1
5E	Goat	Teat Swab	Positive	Negative	Negative	Positive	0	0
6E	Goat	Teat Swab	Positive	Negative	Negative	Positive	0	0
7E	Goat	Teat Swab	Positive	Negative	Negative	Positive	0	0
8E	Goat	Teat Swab	Positive	Positive	Positive	Positive	1	1

[1] #sample = Number+Farm

* 1= Positive; 2= Negative

S. aureus
MR-CoNS
MRSA

Figure 1. Milk sampling in parallel milking parlour