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Original article

Nasal carriage of various staphylococcal species in small ruminant lentivirus-infected asymptomatic goats

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Abstract

The study was carried out in Polish goat population to estimate the prevalence of the nasal cavity infection with various staphylococcal species including methicillin-resistant *Staphylococcus aureus* (MRSA), investigate the potential permissive role of small ruminant lentivirus (SRLV) infection and determine the level of clonality of *S. aureus* nasal isolates. Nasal swabs and blood samples were collected from 1300 clinically healthy adult goats from 21 Polish goat herds. Blood samples were serologically screened for SRLV. Staphylococci were isolated from nasal swabs and identified using classical microbiological methods, MALDI-TOF, multiplex-PCR, and their clonality was assessed using PFGE. Antimicrobial resistance was determined on the basis of minimum inhibitory concentration and by demonstration of the presence of the mecA gene encoding the multiplex-PCR PBP2a protein and of the five main types of staphylococcal cassette chromosome mec. The apparent prevalence of staphylococcal and *S. aureus* infection of the nasal cavity was 29.1% (CI 95%: 26.9%, 31.5%) and 7.3% (CI 95%: 6.1%, 8.8%), respectively. No relationship was found between the SRLV-infection and the presence of any staphylococcal species including *S. aureus* (p=0.143). Only 9.8% of *S. aureus* isolates were resistant to amoxicillin/clavulanic acid and 5.9% to chloramphenicol and ciprofloxacin. All tested isolates proved to be phenotypically and genotypically sensitive to methicillin, which yielded the apparent prevalence of MRSA of 0% (CI 95%: 0%, 7.0%). *S. aureus* isolates show high genetic similarity within goat herds, however vary considerably between herds. Goats do not appear to be an important source of *S. aureus* for humans in Poland.

Key words: caprine arthritis-encephalitis, MALDI-TOF, PFGE, *Staphylococcus aureus*

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Introduction

Staphylococci tend to colonize nasal cavity in people and various animal species (Sakr et al. 2018) including small ruminants (Rahimi et al. 2015). Even though, primarily, they are commensals (Krismer et al. 2017), nasal carriage of staphylococci predisposes to distant localized or generalized infections (Eiff et al. 2001). Especially dangerous are infections caused by methicillin-resistant strains of *Staphylococcus aureus* (MRSA) (Defres et al. 2009), nowadays often called multi-drug resistant, which signifies resistance to more than three groups of chemotherapeutics. MRSA strains have been frequently isolated from companion and farm animals (Baptiste et al. 2005, Burstiner et al. 2010, Cuny et al. 2010). Some studies have also indicated animal-to--human transmission of staphylococci (Loeffler et al. 2005, Faires et al. 2009). Interspecies transmission may be facilitated by factors promoting proliferation of staphylococci in the airways. In humans these factors are known to include infections causing immunosuppression (Sabbagh et al. 2018) and chronic inflammatory diseases of the respiratory system (Kim et al. 2018).

Small ruminant lentivirus (SRLV) causes a progressive disease of goats and sheep. Contrary to human, bovine and feline lentiviruses (SRLV) appear not to have immunosuppressive potential (Ramirez et al. 2013), however chronic interstitial pneumonia, which develops in sheep and, to a lesser extent, also in goats (Ellis et al. 1988), might foster opportunistic bacterial infections of the respiratory tract. Therefore, we carried out the study in Polish goat population to estimate the prevalence of the nasal cavity infection with various staphylococcal species including MRSA, investigate the potential predisposing role of SRLV infection and determine the level of clonality of *S. aureus* nasal isolates.

Materials and Methods

Nasal swabs and blood samples were collected from 1300 clinically healthy adult goats (older than one year) between June and August 2014. The animals came from 21 Polish goat herds.

Blood samples were incubated overnight at +4°C and then centrifuged at 3000 rpm for 10 min. Serum was harvested, aliquoted into 2 ml plastic vials and stored at -20°C until testing. Serum samples were screened for small ruminant lentivirus (SRLV) infection using ID Screen MVV-CAEV Indirect Screening test (ID.vet Innovative Diagnostics, Grabels, France). The assay was performed according to manufacturer's manual and a result was considered positive if a sample-to-positive optical density control ratio was >50%.

Nasal swabs were incubated at 37°C for 24h first in Muller-Hinton medium supplemented with 6.5% sodium chloride and then on Columbia agar supplemented with 5% blood. Classical microbiological phenotypic characterization of colony morphology as well as the presence and type of haemolysis, coagulase production (slide and tube tests with coagulase), catalase production (tests with hydrogen peroxide), determination of staphylococcal clumping factor, were used to assign isolates to the *Staphylococcus* genus.

Mass spectra of those isolates were measured using the mass spectrometer MALDI-TOF ultrafleXtrme (Bruker Daltonics GmbH, Bremen, Germany). An isolated colony was deposited on a steel target plate. Then the deposited bacteria were overlaid with 1 µl solution (α-cyano-4-hydroxycinnamic acid in 50% acetonitrile and 2.5% trifluoroacetic acid) and air-dried at room temperature to allow co-crystallization with the experimental sample. Spectra were recorded in the positive linear mode for a mass range of 2000 to 20000 Da (laser frequency 200 Hz; ion source voltage one, 25 kV; ion source voltage two, 23.5 kV; lens voltage, 6.0 kV). Each spectrum was obtained by averaging 1500 laser shots acquired from three spot positions under control of flexControl software 3.4 (Bruker Daltonics GmbH, Bremen, Germany). For the bacterial identification, Biotyper 3.1 software (Bruker Daltonics GmbH, Bremen, Germany) and a database containing 4613 entries were applied. The following identification criteria were applied depending on the confidence interval of score value: 2.300-3.000 – highly probable species identification; 2.000-2.299 – probable species identification; 1.700-1.999 – probable genus identification; 0.000-1.699 – non-reliable identification.

DNA from strains were isolated using the Wizard Genomic DNA Purification Kit (Promega, Madison, USA) following the instructions provided by the manufacturer with minor modifications concerning the use of lytic enzyme (cells lysed using 10 mg/ml lysostaphin). Samples were stored at -20°C until testing.

Seven pairs of primers (synthetized by Genomed, Warsaw, Poland, according to the method described by Sasaki et al. 2010) allowing the identification of *S. aureus*, *S. intermedius*, *S. schleiferi* subsp. *coagulans*, *S. schleiferi* subsp. *schleiferi*, *S. delphini* group A and group B, *S. hyicus*, *S. pseudintermedius*, were used (Table 1).

The reaction mixture for PCR consisted of 1 µl of DNA extract, 6.3 µl of H_2O, 2 µl of dNTP, 4 µl of buffer, 0.4 µl of primers, 1 µl of $MgCl_2$, and 0.1 µl of polymerase. The reaction mixtures were thermally cycled once at 95°C for 2 min; 30 times at 95°C for 30 s, 56°C for 35 s, and 72°C for 1 min; and finally once at 72°C for 2 min. DNA fragments were analysed

Table 1. Oligonucleotide primers used in the multiplex-PCR for species identification (Sasaki et al. 2010).

Name of primer	Sequence (5' - 3')	Size of PCR product (bp)	Identified species
au-F3 au-nucR	TCGCTTGCTATGATTGTGG GCCAATGTTCTACCATAGC	359	*S. aureus*
in-F in-R3	CATGTCATATTATTGCGAATGA AGGACCATCACCATTGACAT	430	*S. intermedius*
sch-F sch-R	AATGGCTACAATGATAATCACTAA CATATCTGTCTTTCGGCGCG	526	*S. schleiferi* subsp. *coagulans* *S. schleiferi* subsp. *schleiferi*
dea-F dea-R	TGAAGGCATATTGTAGAACAA CGRTACTTTTCGTTAGGTCG	661	*S. delphini* group A
hy-F1 hy-R1	CATTATATGATTTGAACGTG GAATCAATATCGTAAAGTTGC	793	*S. hyicus*
pse-F2 pse-R5	TRGGCAGTAGGATTCGTTAA CTTTTGTGCTYCMTTTTGG	926	*S. pseudintermedius*
deb-F deb-R4	GGAAGRTTCGTTTTTCCTAGAC TATGCGATTCAAGAACTGA	1135	*S. delphini* group B

by electrophoresis in 0.5x Tris-Borate-EDTA buffer on a 2% agarose gel and visualised under UV light after SimplySafe (EURx, Gdańsk, Poland) staining.

Methicillin resistance was confirmed by demonstrating the presence of the mecA gene encoding the multiplex-PCR PBP2a protein and of the five main types of staphylococcal cassette chromosome mec (SCCmec) multiplex-PCR, according to the method described by Kondo et al. (2007).

For all isolates identified as *S. aureus* in PFGE analysis the determination of the minimum inhibitory concentration (MIC) for antibiotics from the main antimicrobial groups (i.e. amoxicillin/clavulanic acid, cephalothin, ciprofloxacin, clindamycin, gentamycin, chloramphenicol, mupirocin) was performed.

To determine the genetic similarity of strains from goats the electrophoretic method of decomposition of DNA fragments in a variable electric field (PFGE) was performed. PFGE genotyping was performed according to the procedure of "Unified Pulsed-Field Gel Electrophoresis (PFGE) Protocol for Gram Positive Bacteria" (08/2012) with its own modifications. After a 24-hour incubation on TSA medium, supplemented with 5% sheep blood, the bacterial colonies were re-suspended in 1 x TE buffer (10 mM Tris-1 mM EDTA, pH 8.0, EURx, Gdańsk, Poland) to a value of 8.0 on McFarland's scale. The relevant number of 1.5 ml sterile tubes were prepared and 20 µl lysozyme (20 mg/ml, Sigma-Aldrich, Poznań, Poland) and 5 µl lysostaphin (1 mg/ml in 20 mM sodium acetate, EURx, Gdańsk, Poland) were added to each. Next, 400 µl of the bacterial suspension was transferred to the tubes and incubated for 30-45 minutes at 55°C in a water bath. The prepared suspension was combined at a 2:1 ratio with pre-dissolved 1% low-melting point agarose (Low-Melting-Point agarose, Bio-Rad, Warsaw, Poland), then transferred to cuboidal moulds and incubated for 20 min on ice. The prepared blocks were incubated in 495 µl of lysis buffer (50 mM Tris: 50 mM EDTA: 1% sarcosyl, EURx, Gdańsk, Poland) with the addition of 5 µl of proteinase K (10 mg/ml, Sigma-Aldrich, Poznań, Poland) at 50°C overnight. After incubation, the blocks were washed in 500 µl of water for 15-20 min, 3 times in 500 µl 1xTE buffer for 10-15 min and once in restriction buffer 10x ONE Buffer (EURx, Gdańsk, Poland) for 15 min in a hybridization oven at the room temperature. The tested DNA was digested with restriction enzyme SmaI (10 U/µl, EURx) for 3.5 hours at 25°C. The electrophoresis was performed in a 1% agarose gel in a CHEF DR III apparatus (Bio-Rad, Warsaw, Poland) for 20 hours, using the following conditions: temperature 14°C, 10 × TBE buffer (890 mM Tris, 890 mM boric acid and 20 mM EDTA, EURx, Gdańsk, Poland), voltage 6 V/cm, length of pulses from 5 s to 40 s. A marker sized ProMega-Markers® Lambda Ladders (Promega, Madison, USA) was used. The gel was stained with ethidium bromide (10 µg/ml, Sigma-Aldrich, Poznań, Poland) for 30 min. The result of the electrophoretic separation was documented with the Gel Doc 1000 Gel Documentation System (Bio-Rad, Warsaw, Poland). The ProMega-Markers® Lambda Ladders marker (Promega, Madison, USA) was used in the electrophoretic section. PFGE genotyping results were analysed using BioNumerics software (Applied Maths, bioMérieux, Schaerbeek, Belgium) using the UPGMA clustering method and the Dice correlation coefficient.

Ninety five percent confidence intervals (CI 95%)

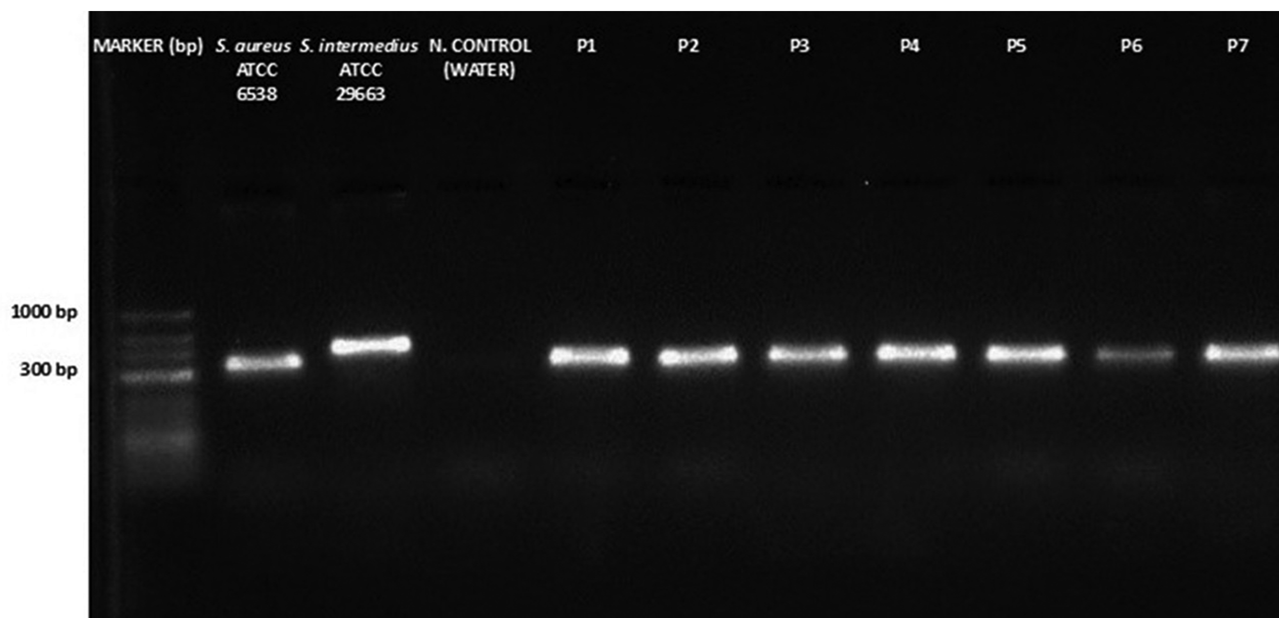

Fig. 1. Electrophoresis of multiplex PCR amplified fragments used for species identification of coagulase-positive staphylococci on a 2% agarose gel. P1-P7 – selected isolates identified as *S. aureus*.

for proportions were calculated using the Wilson's score method (Altman et al. 2000). Relationship between SRLV infection and isolation of the specific staphylococcal species was assessed using the Pearson's chi-square test with the two-tailed significance level (α) of 0.05. Analysis was performed in TIBCO Statistica 13.3.0 (TIBCO Software Inc., California, USA).

Results

Of 1300 collected swabs 515 bacterial isolates were identified as *Staphylococcus* spp. in classical microbiological phenotypic characterization. The MALDI-TOF method allowed for definitive species identification (cut-off score value >2.000) of 437 (84.9%) of them, which resulted in the apparent prevalence of 29.1% (CI 95%: 26.9%, 31.5%). Remaining 78 isolates, whose score value was <2.000, were excluded from further analysis.

Among 437 strains, 178 isolates (40.7%) were identified as *Staphylococcus simulans*, 113 (25.9%) as *S. xylosus*, 110 (25.2%) as *S. aureus*, 12 (2.8%) as *S. cohnii*, 10 (2.3%) as *S. lentus*, 6 (1.4%) as *S. sciuri*, 4 (0.9%) as *S. contimenti*, 2 (0.5%) as *S. arlettae* and 2 (0.5%) as *S. pseudintermedius*. The apparent prevalence of *S. aureus* nasal infection in goats was 7.3% (CI 95%: 6.1%, 8.8%).

Of 437 goats in which staphylococci were isolated from the nasal cavity, 188 (43.0%) were seropositive to SRLV. There was no relationship between the SRLV-infection and the presence of *S. simulans* (p=0.730), *S. xylosus* (p=0.098), or *S. aureus* (p=0.143).

Of 110 isolates classed as *S. aureus* by the MALDI--TOF method, 74 were selected for further analyses. Fifty one of those 74 isolates (68.9%) were confirmed as *S. aureus* using the multiplex-PCR (Fig. 1). They came from goats kept in 5 different herds (from A through E) – 23 isolates from herd D, 21 from herd A, 3 from herd C, another 3 herd E, and 1 from herd B. Based on the PFGE analysis 47 of them could be classed into 9 groups of 100% genetic identity, counting from 2 through 17 isolates (Fig. 2). Isolates from herd C and herd E belonged to two distinct groups. Isolates from herd D to 3 distinct groups, of which 2 (including 21 of 23 isolates from this herd) showed 90% identity. Isolates from herd A belonged to 6 distinct groups, however one group was common with herd D and one with herd E. Three isolates from herd A and one isolate from herd B could not be allocated to any group.

Only 5 of 51 PFGE-confirmed *S. aureus* isolates (9.8%) were resistant to amoxicillin/clavulanic acid and 3 isolates (5.9%) to chloramphenicol and ciprofloxacin. All tested isolates proved to be phenotypically and genotypically sensitive to methicillin, which yielded the apparent prevalence of MRSA in the nasal cavity of goats of 0% (CI 95%: 0%, 7.0%).

Discussion

This study shows that staphylococci inhabit nasal cavity of roughly 30% of adult dairy goats in the study population and according to the MALDI-TOF method more than two thirds of them are coagulase-negative

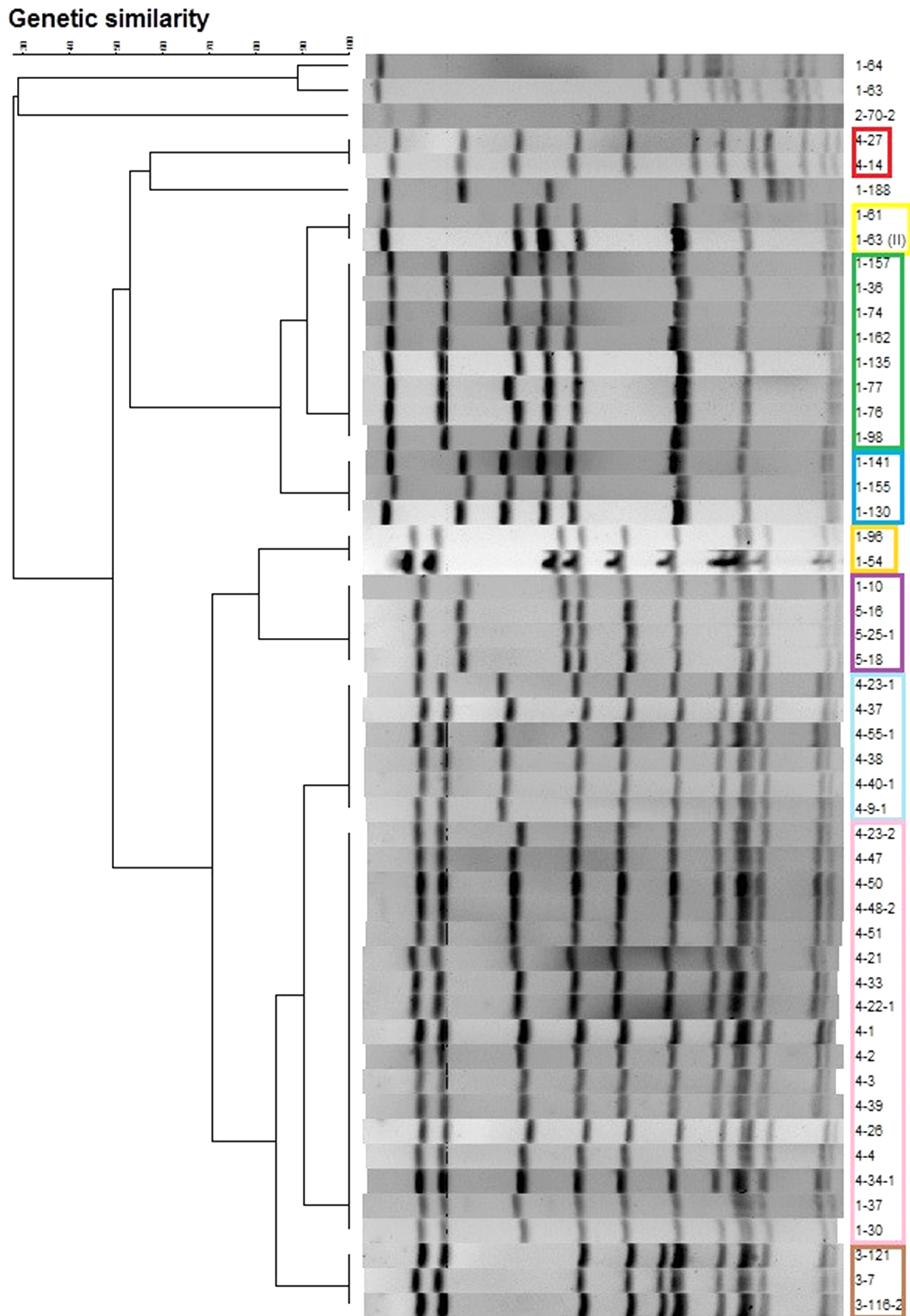

Fig. 2. Results of PFGE genotyping of coagulase-positive *S. aureus* isolated from goat herds in Poland. Herds of origin from A through E are indicated in the first digit (before the hyphen) of the isolate number from 1 through 5, respectively. Color frames indicate identical isolates.

staphylococci. *S. aureus* accounts for only one fourth of them and remains susceptible to the vast majority of antibiotics, including full susceptibility to methicillin. *S. aureus* prevalence observed in this study is low compared to prevalences observed in cattle and sheep in other regions of the world varying from 6% to 70% (Mørk et al. 2010, Gharsa et al. 2015, Rahimi et al. 2015, Agabou et al. 2017, Kumar et al. 2017, Zhou et al. 2017). Low level of general antimicrobial resistance compared to other observations in cattle and small ruminants (Rahimi et al. 2015, Varela-Ortiz et al. 2018) is likely to result from rare use of antibiotics in Polish goat herds. On the other hand, prevalence of nasal carriage of MRSA has been low in all epidemiological studies carried out so far in goats (Chu et al. 2012, El-Deeb et al. 2018, Odetokun et al. 2018). This confirms that goats are very unlikely to pose any important epidemiological threat to humans.

Moreover, colonization of the nasal cavity with various staphylococci does not appear to be enhanced by the infection with SRLV. This is the next evidence against the immunosuppressive potential of this lentivirus. SRLV is known to trigger chronic inflammatory reaction in the lung tissue of infected goats (Ellis et al. 1988), however this reaction is unlikely to affect the microflora of the upper respiratory tract. Therefore, in terms of *S. aureus* transmission, SRLV-infected goats do not appear to pose any higher threat to people than goats free from SRLV.

The MALDI-TOF mass spectrometry is increasingly used in microbiological diagnostics as it requires small amount of material and consumes much less time compared to classical biochemical analyses. However, its credibility is related to the scope of microorganisms available in the database of reference spectra. The MALDI-TOF databases, although constantly updated, do not always allow for accurate species identification, especially due to the fact that they are based on the spectra of the human clinical strains. The difficulties in correct identification of species may therefore result from too low number of representative strains in the databases.

Concluding, even though staphylococci are quite common inhabitants of the nasal cavity of goats, *S. aureus* accounts for only a small proportion of them, both in SRLV-positive and negative herds. *S. aureus* isolates show high genetic similarity within goat herds, however vary considerably between herds. Furthermore, they are generally susceptible to vast majority of antimicrobials. Therefore, goats do not appear to be an important source of *S. aureus* for humans in Poland.

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References

Agabou A, Ouchenane Z, Ngba Essebe C, Khemissi S, Chehboub MT, Chehboub IB, Sotto A, Dunyach-Remy C, Lavigne JP (**2017**) Emergence of nasal carriage of ST80 and ST152 PVL+ *Staphylococcus aureus* isolates from livestock in Algeria. Toxins (Basel) 9: E303.

Altman D, Machin D, Bryant T, Gardner M (**2000**) Statistics with confidence: confidence intervals and statistical guidelines. 2nd ed., BMJ Books, London.

Baptiste KE, Williams K, Williams NJ, Wattret A, Clegg PD, Dawson S, Corkill JE, O'Neill T, Hart CA (**2005**) Methicillin-resistant staphylococci in companion animals. Emerg Infect Dis 11: 1942-1944.

Burstiner LC, Faires M, Weese JS (**2010**) Methicillin-resistant *Staphylococcus aureus* colonization in personnel attending a veterinary surgery conference. Vet Surg 39: 150-157.

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.

Cuny C, Friedrich A, Kozytska S, Layer F, Nübel U, Ohlsen K, Strommenger B, Walther B, Wieler L, Witte W (**2010**) Emergence of methicillin-resistant *Staphylococcus aureus* (MRSA) in different animal species. Int J Med Microbiol 300: 109-117.

Defres S, Marwick C, Nathwani D (**2009**) MRSA as a cause of lung infection including airway infection, community-acquired pneumonia and hospital-acquired pneumonia. Eur Respir J 34: 1470-1476.

El-Deeb W, Fayez M, Elmoslemany A, Kandeel M, Zidan K (**2018**) Methicillin resistant *Staphylococcus aureus* among goat farms in Eastern province, Saudi Arabia: Prevalence and risk factors. Prev Vet Med 156: 84-90.

Ellis TM, Robinson WF, Wilcox GE (**1988**) The pathology and aetiology of lung lesions in goats infected with caprine arthritis-encephalitis virus. Aust Vet J 65: 69-73.

Faires MC, Tater KC, Weese JS (**2009**) An investigation of methicillin-resistant *Staphylococcus aureus* colonization in people and pets in the same household with an infected person or infected pet. J Am Vet Med Assoc 235: 540-543.

Gharsa H, Ben Slama K, Gómez-Sanz E, Lozano C, Zarazaga M, Messadi L, Boudabous A, Torres C (**2015**) Molecular characterization of *Staphylococcus aureus* from nasal samples of healthy farm animals and pets in Tunisia. Vector Borne Zoonotic Dis 15: 109-115.

Kim MH, Choi SJ, Choi HI, Choi JP, Park HK, Kim EK,

Kim MJ, Moon BS, Min TK, Rho M, Cho YJ, Yang S, Kim YK, Kim YY, Pyun BY (**2018**) Lactobacillus plantarum-derived Extracellular Vesicles Protect Atopic Dermatitis Induced by *Staphylococcus aureus*-derived Extracellular Vesicles. Allergy Asthma Immunol Res 10: 516-532.

Krismer B, Weidenmaier C, Zipperer A, Peschel A (**2017**) The commensal lifestyle of *Staphylococcus aureus* and its interactions with the nasal microbiota. Nat Rev Microbiol 15: 675-687.

Kumar A, Kaushik P, Anjay, Kumar P, Kumar M (**2017**) Prevalence of methicillin-resistant *Staphylococcus aureus* skin and nasal carriage isolates from bovines and its antibiogram. Vet World 10: 593-597.

Loeffler A, Boag AK, Sung J, Lindsay JA, Guardabassi L, Dalsgaard A, Smith H, Stevens KB, Lloyd DH (**2005**) Prevalence of methicillin-resistant *Staphylococcus aureus* among staff and pets in a small animal referral hospital in the UK. J Antimicrob Chemother 56: 692-697.

Mørk T, Kvitle B, Mathisen T, Jørgensen HJ (**2010**) Bacteriological and molecular investigations of *Staphylococcus aureus* in dairy goats. Vet Microbiol 141: 134-141.

Odetokun IA, Ballhausen B, Adetunji VO, Ghali-Mohammed I, Adelowo MT, Adetunji SA, Fetsch A (**2018**) *Staphylococcus aureus* in two municipal abattoirs in Nigeria: Risk perception, spread and public health implications. Vet Microbiol 216: 52-59.

Rahimi H, Dastmalchi Saei H, Ahmadi M (**2015**) Nasal Carriage of *Staphylococcus aureus*: Frequency and Antibiotic Resistance in Healthy Ruminants. Jundishapur J Microbiol 8: e22413.

Ramírez H, Reina R, Amorena B, de Andrés D, Martínez HA (**2013**) Small ruminant lentiviruses: genetic variability, tropism and diagnosis. Viruses 5: 1175-1207.

Sabbagh P, Riahi SM, Gamble HR, Rostami A (**2018**) The global and regional prevalence, burden, and risk factors for methicillin-resistant *Staphylococcus aureus* colonization in HIV-infected people: A systematic review and meta-analysis. Am J Infect Control 47: 323-333

Sakr A, Brégeon F, Mège JL, Rolain JM, Blin O (**2018**) *Staphylococcus aureus* Nasal Colonization: An Update on Mechanisms, Epidemiology, Risk Factors, and Subsequent Infections. Front Microbiol 9: 2419.

Sasaki T, Tsubakishita S, Tanaka Y, Sakusabe A, Ohtsuka M, Hirotaki S, Kawakami T, Fukata T, Hiramatsu K (**2010**) Multiplex-PCR method for species identification of coagulase-positive staphylococci. J Clin Microbiol 48: 765-769.

Varela-Ortiz DF, Barboza-Corona JE, González-Marrero J, León-Galván MF, Valencia-Posadas M, Lechuga--Arana AA, Sánchez-Felipe CG, Ledezma-García F, Gutiérrez-Chávez AJ (**2018**) Antibiotic susceptibility of *Staphylococcus aureus* isolated from subclinical bovine mastitis cases and in vitro efficacy of bacteriophage. Vet Res Commun 42: 243-250.

von Eiff C, Becker K, Machka K, Stammer H, Peters G (2001) Nasal carriage as a source of Staphylococcus aureus bacteremia. Study Group. N Engl J Med 344: 11-16.

Zhou Z, Zhang M, Li H, Yang H, Li X, Song X, Wang Z (**2017**) Prevalence and molecular characterization of Staphylococcus aureus isolated from goats in Chongqing, China. BMC Vet Res 13: 352.