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Transmission of MRSA between humans and animals on duck and turkey farms

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Objectives: The objectives of this study were to estimate the prevalence of MRSA on duck and turkey farms, identify risk factors for human carriage and study transmission between animals and humans.

Methods: On 10 duck and 10 turkey farms, samples were taken from animals, poultry houses, home residences and humans and cultured using pre-enrichment and selective enrichment. MRSA isolates were typed by *spa* typing and multiple-locus variable number tandem repeat analysis (MLVA) typing. A subset of isolates from animals and humans was investigated using whole-genome mapping.

Results: MRSA was found on one duck farm and three turkey farms. On duck farms, all humans were MRSA negative. On turkey farms, 5 of 11 farmers, 2 of 32 family members and 15 of 49 samples from the home residences were MRSA positive. Individuals with daily contact with turkeys were significantly more often MRSA positive than individuals without daily contact. All MRSA isolates belonged to livestock-associated MLVA complex 398, belonged to *spa* type t011, were negative for Panton–Valentine leucocidin, were *mecC* negative and were *mecA* positive. Whole-genome mapping proved a valuable tool to study the transmission of livestock-associated MRSA and showed that on two turkey farms the isolates from the animals and humans were indistinguishable or closely related, indicating transmission.

Conclusions: MRSA carriage in individuals in daily contact with turkeys was significantly higher than that in individuals only living on the farms or than in the general Dutch population. Therefore, persons with a high degree of contact with turkeys have an increased risk of MRSA carriage, and we propose that they should be screened prior to hospitalization in order to decrease the risk of nosocomial transmission.

Introduction

In Europe, livestock-associated MRSA (LA-MRSA) mainly comprises isolates belonging to MLST ST398. MLST ST398 corresponds to multiple-locus variable number tandem repeat analysis (MLVA) complex 398. LA-MRSA can be transmitted from food-producing animals to humans, especially to persons in close contact with animals.[1,2] In the Netherlands, the prevalence of LA-MRSA has been investigated in healthy pigs, veal calves, horses and broilers.[3–7] Fifty-six percent of pig herds were classified as MRSA positive, while MRSA prevalence was 88% on veal calf farms and 16% among persons living and/or working on these farms were MRSA positive.[3,6] LA-MRSA was found on 4 of 50 conventional broiler farms and in 8 of 145 persons (5.5%) living and/or working on these farms. Contact with broilers was identified as a risk factor for MRSA carriage.[5] On eight organic broiler farms, however, no MRSA was detected in humans or animals.[8] In comparison with conventional farms, organic broiler farms have more restrictions on antimicrobial use. In Germany, MRSA has been found in turkeys, in turkey meat and in environmental samples taken inside and outside of turkey farms.[9–12] LA-MRSA—in particular, isolates of clonal complexes (CCs) 398 and 9—have been identified in healthy as well as in diseased turkeys.[13,14] Information on the prevalence of MRSA on commercial duck farms is lacking. On duck farms, smaller quantities of antimicrobials are used than on turkey farms and, therefore, the selective pressure for resistant bacteria is lower. It is likely that LA-MRSA are present in commercially reared poultry other than broilers and may also be transferred to humans who are occupationally exposed to turkeys or ducks. To date, data on the prevalence of MRSA on Dutch duck and turkey farms are lacking. In addition, the prevalence of and risk factors for MRSA carriage in farmers, their family members and the employees on these farms have not yet been studied. According to the Dutch MRSA guideline, persons who have occupational contact with pigs, veal calves or broilers have a higher risk of being MRSA positive and are screened before hospitalization, while persons who have contact with turkeys or ducks are not screened.

The objectives of the present study were to investigate the prevalence of MRSA on Dutch duck and turkey farms and among farmers and their family members, to identify risk factors and to study transmission between animals and humans.

Materials and methods

Sample collection and questionnaires

A cross-sectional MRSA-prevalence survey was conducted on 10 duck farms and 10 turkey farms between November 2013 and September 2014. Samples were collected by a single employee of the Animal Health Service. Duck flocks were sampled from the age of 22 days onwards, and turkey flocks were sampled between the ages of 6 and 15 weeks. On each farm, a single randomly chosen flock was sampled by swabbing the throats of 60 animals (pooled into 10 sample groups). In addition, samples of dust from five locations within all poultry houses—the drinking system at the front and the back of the poultry house, the feeding system at the front and the back of the poultry house and the ventilation system—were taken using Sodibox wipes (sterile cloth with Ringer's solution; Sodibox, France). Inside each farm residence, environmental samples were taken from an armchair, a television remote control, inside and outside door handles and the coat of the pet dog, if present, using Sodibox wipes (sterile cloth with Ringer's solution). People who voluntarily participated in the study took a nose swab for MRSA detection. Farmers completed a questionnaire about farm management, while farmers, family members and employees completed a separate questionnaire on lifestyle and health characteristics. The study was performed according to the Dutch law on studies with animals and humans. Written informed consent was obtained from each participant.

Microbiological analysis

Swab samples were incubated in 10 mL and wipes were incubated in 100 mL of Mueller–Hinton enrichment broth (BD, France) with 6.5% sodium chloride for 18 h at 37°C. For selective enrichment, 1 mL of broth was transferred to 9 mL of phenol red mannitol broth with 5 mg/L ceftizoxime and 75 mg/L aztreonam (bioMérieux, France), incubated for 18 h at 37°C and subsequently plated onto Columbia agar with 5% sheep blood (Oxoid, Germany) and Brilliance MRSA 2 agar (Oxoid, Germany) and incubated for 18 h at 37°C.

Suspected colonies were typed by *spa* typing and MLVA typing as described previously.[15,16] The MLVA for *S. aureus* also included markers for the presence of the *mecA* and *mecC* genes and the *lukF* genes encoding Panton–Valentine leucocidin (PVL).[17]

A farm was classified as MRSA positive if at least a single broiler sample or dust sample from the poultry house tested positive for MRSA. Forty MRSA isolates from ducks ($n=1$), turkeys ($n=15$), humans ($n=7$) and the environments of the poultry houses ($n=12$) and home residences ($n=5$), representing isolates from all MRSA-positive farms, were selected and tested for their susceptibility to tetracycline, linezolid, quinupristin/dalfopristin, vancomycin, clindamycin, erythromycin, gentamicin, ciprofloxacin and trimethoprim/sulfamethoxazole using Etest (bioMérieux, France) and EUCAST breakpoints. *S. aureus* ATCC 29213 served as a quality-control strain.

A subset of 10 isolates from humans and turkeys from the three farms with both positive human and turkey samples was analysed by whole-genome mapping. This method creates high-resolution, ordered whole-genome restriction maps.[18] High molecular weight DNA, with an average molecule size of ~250000 bp, was isolated using the Argus™ HMW DNA isolation kit (OpGen, USA). Thereafter, DNA was applied to MapCards containing microchannels in which DNA molecules were stretched, immobilized to a glass surface, digested with AflII and stained with a fluorescent agent in a microfluids system. The resulting restriction fragments were sized in a whole-genome mapper and assembled into a whole-genome map in which the restriction sites were mapped in the order in which they occur in the chromosome. BioNumerics software version 7.1 (Applied Maths, Belgium) was used for the analysis and clustering of the whole-genome maps. The whole-genome mapping cut-off value for isolates with indistinguishable whole-genome maps was >98%. Isolates with 95%–98% resemblance were considered closely related. Isolates with <95% resemblance were classified as unrelated.[18]

Analysis of risk factors

Questionnaires were analysed only for the turkey farms, as all humans on duck farms were MRSA negative. The frequency of exposure to potential risk factors was calculated for MRSA-positive and MRSA-negative individuals. Fisher's exact test was used to test whether differences in frequencies were significant.

Results

Animals and environment

The median number of animals per farm was 18020 (range 4100–47000) on duck farms and 17940 (range 14800–25340) on turkey farms. On the duck farms, the Cherry Valley breed and/or Peking breed were present, while on turkey farms the B.U.T.6 breed predominated. On duck farms, dust samples were collected from one to three poultry houses and on turkey farms from two to six poultry houses. The MRSA prevalence on duck farms was 10%. MRSA was found in one throat-pool sample and in one dust sample from the poultry house on a single farm. None of the environmental samples from the duck farm residences was MRSA positive (Table 1). MRSA was found on three turkey farms, yielding a prevalence of 30%. MRSA was detected in 17 throat-pool samples (17%) and in 31 of the dust samples from the poultry houses (17%). In 15 of 49 samples from the home residences on turkey farms (31%), MRSA was detected (Table 1).

Humans

In total, 86 persons were working and/or living on the 20 farms: 43 on the duck farms and 43 on the turkey farms. Of these, 69 persons agreed to participate: 26 on duck farms and 43 on turkey

Table 1. Prevalence of MRSA on duck and turkey farms

	Prevalence of MRSA by sample source, % (number of MRSA-positive samples/total number of samples investigated)				
	farms	throat swab pool samples	dust samples, poultry houses	nasal swabs, humans	environment, home residences
Duck farms	10 (1/10)	1 (1/100)	1.3 (1/80)	0.0 (0/26)	0.0 (0/50)
Turkey farms	30 (3/10)	17 (17/100)	17 (31/185)	16 (7/43)	31 (15/49)

Table 2. Results from the questionnaires from the farmers and their family members on turkey farms

Variable	MRSA positive, $n=7$, % (number of participants who answered yes/total number of participants)	MRSA negative, $n=36$, % (number of participants who answered yes/total number of participants)	*P* (Fisher's exact test)
Visiting the poultry houses at least 2 times a day	85.7 (6/7)	16.7 (6/36)	0.001
Physical contact with turkeys at least 2 times a day	85.7 (6/7)	19.4 (7/36)	0.002
Working in healthcare	0.0 (0/7)	5.9 (2/34)	1.00
Antimicrobial therapy during the past 3 months	0.0 (0/7)	20.0 (7/35)	0.326
Hospitalization during the past year	14.3 (1/7)	8.3 (3/36)	0.523
Travelling abroad during the past year	85.7 (6/7)	52.8 (19/36)	0.209

farms. No MRSA-positive persons were found on the duck farms. On 5 of 10 turkey farms, including all three farms with positive animals, one or more individuals tested MRSA positive, and on all of these farms, samples from the home residences were also MRSA positive. On the turkey farms, 5 of 11 farmers (45.5%) were MRSA positive, compared with 2 of 32 family members and employees (6.3%). This difference was statistically significant ($P=0.008$). On the three MRSA-positive turkey farms, 4 of 10 individuals (40%) were MRSA carriers, compared with 3 of 33 individuals (9.1%) on the seven farms where all samples from the turkeys and poultry houses were MRSA negative. The prevalence of MRSA-positive and MRSA-negative persons related to several possible risk factors is shown in Table 2. Visiting the poultry houses and physical contact with turkeys were identified as risk factors for MRSA carriage ($P=0.001-0.002$), while antimicrobial therapy within the past 3 months, working in healthcare, hospitalization during the past year and travelling abroad during the past year could not be identified as risk factors.

All MRSA isolates from animals, humans and the environment were *mecA* positive and *mecC* negative, belonged to MLVA complex 398, were PVL negative and belonged to *spa* type t011. All 40 isolates were resistant to tetracycline and susceptible to linezolid, vancomycin, quinupristin/dalfopristin and trimethoprim/sulfamethoxazole. Resistance to clindamycin and erythromycin was common: 60% of all isolates were resistant to both antimicrobials. More than half of the isolates (52.5%) were resistant to ciprofloxacin, and resistance to gentamicin was also common (52.5%). Both isolates from the duck farm were resistant only to tetracycline. The isolates from turkey farm 1 were resistant to tetracycline, gentamicin, clindamycin and erythromycin, except for one turkey isolate that was only resistant to tetracycline and gentamicin. All isolates from turkey farm 6 were resistant to tetracycline and ciprofloxacin, and six isolates had additional resistances to clindamycin, erythromycin and gentamicin. On turkey farm 10, the isolates from the turkeys and the dust in the stables were resistant to tetracycline and ciprofloxacin only, whereas the isolates from the farmer and the home residence were resistant to tetracycline, clindamycin, erythromycin and gentamicin, but susceptible to ciprofloxacin. On turkey farm 4, the isolates from the farmer and the home residence were resistant to tetracycline, clindamycin and erythromycin, while the isolate from the family member was also resistant to gentamicin. On turkey farm 5, the isolates from the farmer and the home residence were resistant to tetracycline, clindamycin, erythromycin and ciprofloxacin. Whole-genome mapping showed that the isolates from the farmer and the animals on farm 10 were different (75% homology), while the whole-genome maps of the two isolates from the animals on this farm were indistinguishable (>98% similarity). On farm 1, whole-genome maps of the isolates from animals and humans were highly related (>95% homology), while indistinguishable whole-genome maps (>99% similarity) were obtained from the isolates from humans and animals on farm 6. Therefore, on farms 1 and 6, whole-genome maps indicate that transmission occurred between humans and animals, while on farm 10, transmission was not confirmed (Figure 1).

Discussion

To the best of our knowledge, this is the first study on the prevalence of MRSA on duck farms. The MRSA prevalence on duck farms seems lower than on turkey farms: fewer farms and fewer samples were found positive. This difference might be due to the lower consumption of antimicrobials on duck farms compared with turkey farms, but other explanations, e.g. different management practices, are also possible. Richter *et al.*[11] found that 18 of 20 of the flocks investigated (90%) were MRSA positive on German turkey farms. Another German study showed that 20% of dust samples on turkey farms, 66% of turkey carcasses and 32% of turkey meat samples were MRSA positive.[12] Friese *et al.*[10] were able to detect MRSA in the air outside of two turkey farms and in the soil of 44% of the turkey and broiler farms investigated. MRSA CC398 can cause infections in animals and has been isolated from an abscess of a turkey.[13] Most isolates belonged to the livestock-associated CC398 (*spa* types t011 and t034), but other *spa* types related to other MRSA lineages, such as t1430 and t002, were also found. In the present study, all isolates belonged to CC398 and *spa* type t011 and were resistant to tetracycline. The MRSA prevalence among animals on turkey farms found in the present study was lower than that found in the study of Richter *et al.*,[11] but higher than that found in the study of Vossenkuhl *et al.*[12] Possible explanations might be the different populations investigated, different management systems on the farms or different methods used between the studies.

Of the 43 persons associated with the turkey farms, 16% tested MRSA positive. On MRSA-positive farms, 4 of 10 individuals (40%) were MRSA positive, compared with 3 of 33 individuals (9.1%) on farms where all samples from the turkeys and the poultry houses were MRSA negative. This finding is much higher than the prevalence in the general population in the Netherlands, which is 0.2%.[19] On conventional broiler farms, 5.5% of persons living and/or working on these farms were found to be MRSA positive.[5]

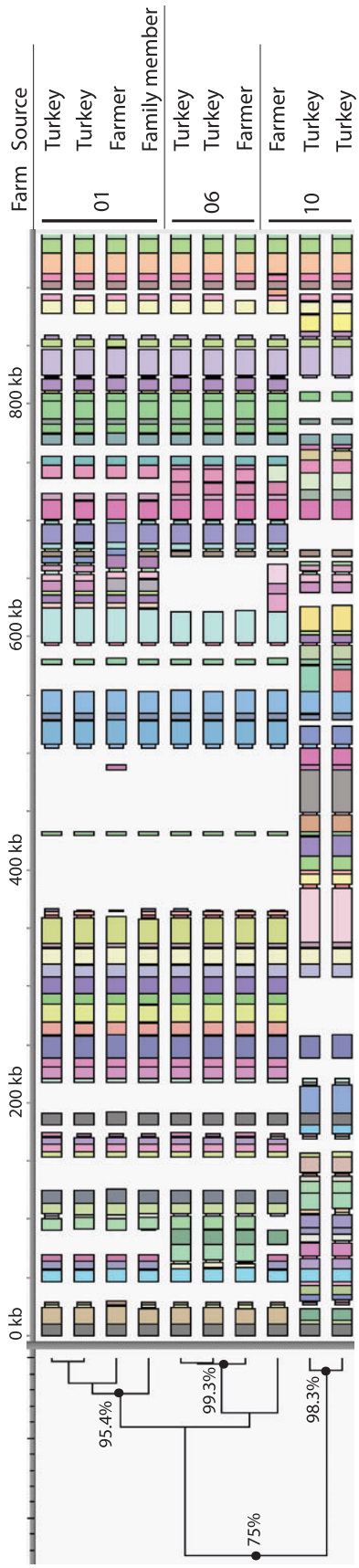

Figure 1. Whole-genome maps of MRSA isolates ($n = 10$) from farmers, a family member and turkeys on three farms (farms 1, 6 and 10). This figure appears in colour in the online version of *JAC* and in black and white in the print version of *JAC*.

Richter *et al.*[11] found an MRSA prevalence of 37% among persons on turkey farms, but, in their study, the MRSA prevalence among the turkey farms was much higher than that in the present study. The human MRSA carriage rate was higher among farmers on Belgian veal farms (72%) than among those on beef farms (11%) or broiler farms (3%).[20] In the present study, farmers were more often carriers than family members, and physical contact with live animals and entering the poultry house were identified as risk factors. Most MRSA-positive individuals were farmers, and both MRSA-positive family members reported frequent contact with turkeys. Whole-genome mapping proved a good tool to investigate LA-MRSA transmission, as all isolates in the present study had the same MLVA type and *spa* type. *spa* typing and MLVA typing are often used to investigate transmission of LA-MRSA, but although this strategy is well suited for characterizing most MRSA isolates, these methods provide very low discriminatory power for isolates belonging to MLVA complex 398. In the present study, isolates with the same *spa* type and MLVA type were sometimes found to be only distantly related using whole-genome mapping, indicating that they were not epidemiologically related. Whole-genome mapping has much higher resolution, and, using this method, epidemiologically unrelated LA-MRSA isolates that were previously indistinguishable by *spa* typing and MLVA typing can be differentiated. Other methods, including microarrays, PFGE and susceptibility testing can also be used. Transmission between animals and humans was likely on two out of three farms. On the other MRSA-positive farm, isolates from the animals differed from that of the farmer: the resistance patterns and the whole-genome maps were different. These findings are in accordance with the results from a previous study of MRSA on dairy farms.[21] The farmer might have been colonized from a source outside the farm, or from a different batch of animals, as not all animals present on the farm were investigated; turkeys from previous production rounds might also have been the source. This explanation might also account for the MRSA-positive individuals on two farms with MRSA-negative animals. Contact with pigs, veal calves or broilers has been previously identified as a risk factor for MRSA carriage.[2,5,22] The home environment in households with MRSA-positive persons was often contaminated with MRSA, and therefore the environment might be a source of (re)infection for persons in the household.

In conclusion, people in contact with turkeys should be considered a risk group for nasal colonization with MRSA and should be screened at admission to healthcare facilities in order to minimize the possible entry of resistant bacteria in hospitals and thus potential risks to patients and staff.

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Transparency declarations

None to declare.

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