Transmission and Persistence of Livestock-Associated Methicillin-Resistant *Staphylococcus aureus* among Veterinarians and Their Household Members

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After the first isolation of livestock-associated methicillin-resistant *Staphylococcus aureus* (LA-MRSA) in 2003, this MRSA variant quickly became the predominant MRSA obtained from humans as part of the Dutch national MRSA surveillance. Previous studies have suggested that human-to-human transmission of LA-MRSA, compared to that of other MRSA lineages, rarely occurs. However, these reports describe the transmission of LA-MRSA based on epidemiology and limited molecular characterization of isolates, making it difficult to assess whether transmission actually occurred. In this study, we used whole-genome maps (WGMs) to identify possible transmission of LA-MRSA between humans. For this, we used LA-MRSA isolates originating from a 2-year prospective longitudinal cohort study in which livestock veterinarians and their household members were repeatedly sampled for the presence of *S. aureus*. A considerable degree of genotypic variation among LA-MRSA strains was observed. However, there was very limited variability between the maps of the isolates originating from the same veterinarian, indicating that each of the veterinarians persistently carried or had reacquired the same LA-MRSA strain. Comparison of WGMs revealed that LA-MRSA transmission had likely occurred within virtually every veterinarian household. Yet only a single LA-MRSA strain per household appeared to be involved in transmission. The results corroborate our previous finding that LA-MRSA is genetically diverse. Furthermore, this study shows that transmission of LA-MRSA between humans occurs and that carriage of LA-MRSA can be persistent, thus posing a potential risk for spread of this highly resistant pathogen in the community.

Shortly after the introduction of methicillin in 1959, methicillin-resistant *Staphylococcus aureus* (MRSA) emerged as an important human pathogen (1). Currently, MRSA is held responsible for numerous hospital-acquired infections worldwide, such as skin infections and toxic shock syndrome (2). MRSA also emerged as a community-acquired pathogen and in recent years has increasingly been isolated from livestock (3, 4). Livestock-associated MRSA (LA-MRSA) can be separated from other MRSA strains, as all strains belong to one multilocus sequence type (MLST) clonal complex. Since the first detection of LA-MRSA in 2003, it has been found in many countries worldwide (5–7). In the Netherlands, LA-MRSA has become quite prominent, making up approximately 40% of all MRSA strains isolated from humans that were sent to the National Institute for Public Health and the Environment (RIVM) for molecular typing as part of the Dutch national MRSA surveillance.

From 2002 to 2007, all Dutch MRSA isolates were typed with pulsed-field gel electrophoresis (PFGE) using SmaI, but because of its labor-intensive character and the subjectivity involved in interpretation, this method was replaced by *spa* typing in 2007 (8, 9). In addition, multilocus variable-number tandem-repeat analysis (MLVA) was introduced for *S. aureus* in 2008 (10–12), and since then all isolates have been characterized by MLVA and *spa* typing.

Although *spa* and MLVA typing are very well suited for characterizing most MRSA isolates, they provide very low discriminatory power for isolates belonging to MLVA complex 398 (MC398) (10, 13). The limited differentiation of MC398 isolates, representing LA-MRSA, has impeded investigations on transmission events and possible outbreaks caused by LA-MRSA.

Transmission of LA-MRSA has been described in multiple reports, suggesting that human-to-human transmission of LA-MRSA is less likely to occur than that of other MRSA lineages (14, 15). However, these reports describe the transmission of the MC398 (or CC398) clade based on epidemiology and limited molecular characterization of isolates, making it difficult to interpret if actual transmission events with LA-MRSA did occur.

Recently, a new high-resolution typing technique for LA-MRSA was introduced named whole-genome mapping (13). Using this method, epidemiologically unrelated LA-MRSA isolates that were previously indistinguishable by *spa* and MLVA typing can now be differentiated. Furthermore, the method is able to identify transmission events between livestock veterinarians and their household, showing its potential as a typing tool for LA-MRSA.

In the study presented here, we further investigated the potential of whole-genome mapping to identify possible transmission of

TABLE 1 Bacterial isolates used in this study

Household by veterinarian identifier	Veterinarians			Household members			
	No. of isolates	Period(s) of persistence (mo.)	WGM cluster(s)	No. of HHM	HHM identifier(s) (no. of isolates)	Period(s) of persistence (mo.)	WGM cluster(s)
VET35	5	2–6	cl-05	4	H1 (1)	None	None
VET45	8	6–14	cl-10	3	H1 (3), H2 (2)	H1: 6–10	cl-10
VET59	6	2–14	cl-01	3	H1 (1)	None	cl-01
VET66	7	0–14	cl-07	2	H1 (1), H2 (3)	H2: 10–14	cl-07
VET78	6	0–10	cl-13	3	H1 (5)	H1: 6–14	cl-13
VET84	7	2–14	cl-02	4	H1 (1), H2 (2)	None	cl-02
VET100	8	0–14	cl-06	3	H1 (1)	None	cl-06
VET155	8	0–14	cl-09	3	H1 (3)	H1: 6–14	cl-09
VET212	9	0–14	cl-04	3	H1 (1)	None	cl-04
VET223	8	0–14	cl-07	1	H1 (3)	H1: 6–10	cl-07
VET226	6	0–2, 6–10	cl-07, cl-08	4	H1 (6), H2 (2)	H1: 2–6 and 6–14; H2: 6–10	cl-07, cl-08
VET247	7	0–14	cl-04	3	H1 (1), H2 (1)	None	cl-04
VET300	7	0–14	cl-02, cl-07	4	H1 (1), H2 (5), H3 (2)	H2: 2–10	cl-07
VET312	6	0–10	cl-03	3	H1 (2)	None	cl-03
VET360	8	2–10	cl-11	1	H1 (2)	H1: 2–6	cl-11
VET361	4	2–6	cl-07, cl-12	3	H1 (1), H2 (1)	None	cl-12

LA-MRSA between humans. To assess this, we used LA-MRSA isolates originating from a 2-year prospective longitudinal cohort study in which livestock veterinarians and their household members were repeatedly sampled for the presence of *S. aureus* ([16], [17]).

MATERIALS AND METHODS

Bacterial isolates and study design. In this study, MRSA belonging to MC398 is defined and referred to as LA-MRSA. We used LA-MRSA isolates collected during a 2-year prospective longitudinal cohort study among 135 Dutch livestock veterinarians and their household members ([16], [17]). Samples were taken at baseline (0 months, $t = 0m$) and at 2 to 3 ($t = 2m$), 6 ($t = 6m$), 10 ($t = 10m$), and 14 ($t = 14m$) months after inclusion. From this study, we selected 161 LA-MRSA isolates from 16 epidemiologically unrelated veterinarians with presumed LA-MRSA transmission to their household members based on MLVA and *spa* typing. Of the 161 LA-MRSA isolates, 110 originated from the veterinarians and 51 were obtained from their household members (Table 1).

All isolates were subjected to molecular characterization using the previously described *spa* typing and MLVA methods ([10], [18]).

Whole-genome mapping of *S. aureus* isolates. Whole-genome maps (WGMs) of the 161 *S. aureus* isolates were created as described before ([13]). Briefly, whole-genome maps were created using DNA that was digested with restriction enzyme AflII in a microfluid system. The resulting restriction fragments were sized in the whole-genome mapper, assembled into a whole-genome map, and subsequently imported into a database of BioNumerics version 7.0 (Applied Maths, Sint-Martens-Latem, Belgium) for further analysis ([13]). WGMs with similarities of ≥98% were considered indistinguishable, LA-MRSA strains with WGMs with similarities between 95% and 98% were classified as highly related strains, and LA-MRSA isolates with maps with a similarity of <95% were regarded as different strains. Minimum spanning trees were created using a similarity matrix, wherein nodes with identical colors represent isolates from the same veterinarian (similarity bin size = 0) and the halos represent complexes based on a similarity cutoff value of ≥98% for indistinguishable WGMs.

Ethics statement. This study was approved by the medical ethics committee of the St. Elisabeth Hospital in Tilburg, the Netherlands (protocol number 0749). All adult subjects provided written informed consent. In case of any child participant, a parent or guardian provided written informed consent on their behalf. The reviewing medical ethics committee approved this consent procedure.

RESULTS

Genetic diversity of LA-MRSA. MLVA-based molecular typing revealed that the 161 LA-MRSA isolates comprised 13 different MLVA types (MTs), of which 159 isolates belonged to MLVA complex 398 (MC398). Although two isolates did not fulfill the MC398 complex assignment criteria, they yielded MLVA types that were strongly related to the MC398 complex MLVA types and therefore were considered to be LA-MRSA.

When a cutoff value of 98% for indistinguishable whole-genome maps was applied on all LA-MRSA isolates originating from 16 veterinarians ($n = 110$), 13 different clusters and 8 singletons were identified (Fig. 1A).

Clustering of the WGMs in a minimum spanning tree revealed that 10 of the 13 clusters contained LA-MRSA isolates obtained from a single veterinarian, whereas three clusters (cl-02, cl-04, and cl-07) yielded isolates originating from multiple veterinarians. Of these three clusters, cl-02 contained all isolates from veterinarian identifier 84 (VET84) and five isolates from VET300. One of the other clusters (cl-04) yielded isolates belonging to two veterinarians (VET212 and VET247). Although isolates from these veterinarians were separated from each other in the dendrogram, the overall similarity between the most distinct maps of the veterinarians in this cluster was 98.2%. The third cluster (cl-07) comprised all isolates of VET223 and VET66 (98.5%), three isolates from VET226 (99.2%), two isolates originating from VET300 (98.9%), and a single isolate from VET361 (98.3%).

The isolates that did not partition into a cluster belonged to six different veterinarians. Half of these singletons (4/8) were grouped with isolates originating from the same veterinarian (2 isolates of VET45 [cl-10], 1 isolate of VET226 [cl-08], and 1 isolate of VET35 [cl-05]). The remaining four singletons clustered within a group of isolates from a different veterinarian.

The genetic diversity of LA-MRSA was not observed with MLVA and *spa* typing, as eight of the 16 veterinarians carried LA-MRSA with identical MLVA and *spa* types (Fig. 1C).

Persistent carriage or reacquisition of LA-MRSA. Although the genetic diversity of LA-MRSA found among the veterinarians

A.

B.

C.
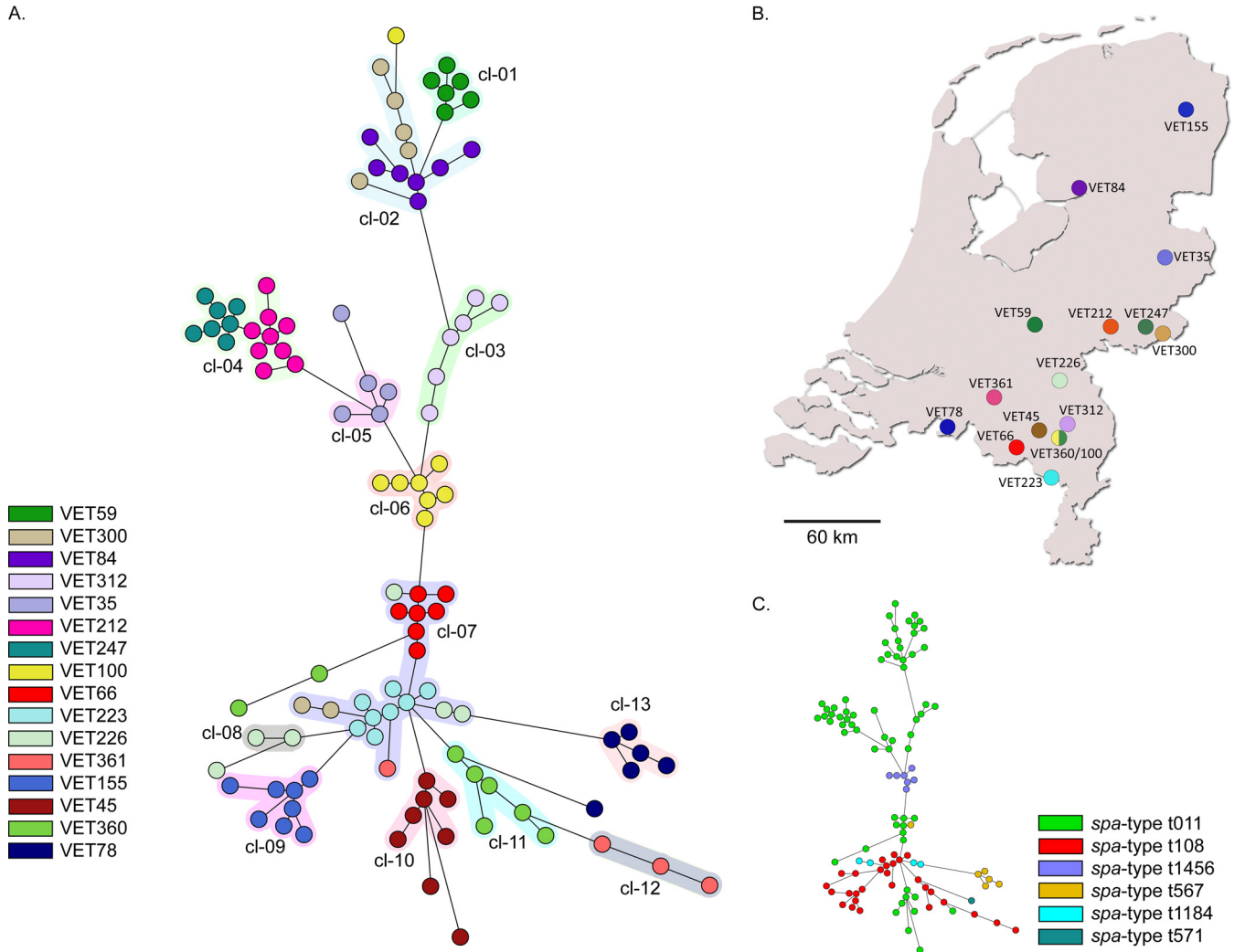
FIG 1 (A) Minimum spanning tree depicting the genotypic diversity among LA-MRSA isolates originating from veterinarians (*n* = 110). Each node in this minimum spanning tree represents the WGM of a single LA-MRSA isolate. Nodes with identical colors represent isolates from the same veterinarian. The halos represent groups based on a similarity cutoff value of ≥98% for indistinguishable WGMs. (B) The demographic location of each veterinarian is represented in the map of the Netherlands. (C) In the smaller version of the minimum spanning tree, the colors of the nodes represent the *spa* types.

in this study was high enough to distinguish different strains, there was very limited variability between the maps of the isolates originating from the same veterinarian. Based on the criterion of indistinguishable WGMs (≥98% similarity), each of the 16 veterinarians persistently carried or had reacquired the same LA-MRSA strain during at least 2 sampling moments, with carriage periods ranging between 4 and 14 months. In eight veterinarians, the LA-MRSA isolates obtained at all sampling moments were indistinguishable, yet each veterinarian carried his own distinct strain. In addition, a single veterinarian likely carried the same LA-MRSA strain at all sampling moments, but the similarities of the most distinct maps among the isolates of the same veterinarian were 96.9% (VET35, cl-05) (Fig. 1). This indicates that, according to our criteria, one of these isolates was a highly related yet distinct strain.

In three other veterinarians, isolates with indistinguishable WGMs were obtained at multiple sampling moments, but for each veterinarian, an isolate with a different map belonging to

a different cluster was found at one of the sampling moments (Fig. 1, VET100, VET361, and VET78). In three of the remaining four veterinarians, two isolates differed from the other isolates originating from the veterinarians (Fig. 1, VET300, VET45, and VET360).

In a single veterinarian (VET226), two distinct LA-MRSA strains were obtained at multiple time points. The first strain was isolated at *t* = 0m and *t* = 2m (cl-07), while the second was isolated at *t* = 6m and at *t* = 10m (cl-08) (Fig. 1, VET226).

Transmission of LA-MRSA. Comparison of WGMs of LA-MRSA revealed that transmission had likely occurred within 14 of the 16 veterinarian households (similarities per household ranged from 98.5% to 100%). In these 14 households, only a single LA-MRSA strain per household appeared to be involved in transmission, and the WGMs of these LA-MRSA strains differed considerably between households (Fig. 2). In addition, in one of the two remaining households (VET35), there was no likely transmission between the veterinarian and his household member; the similar-

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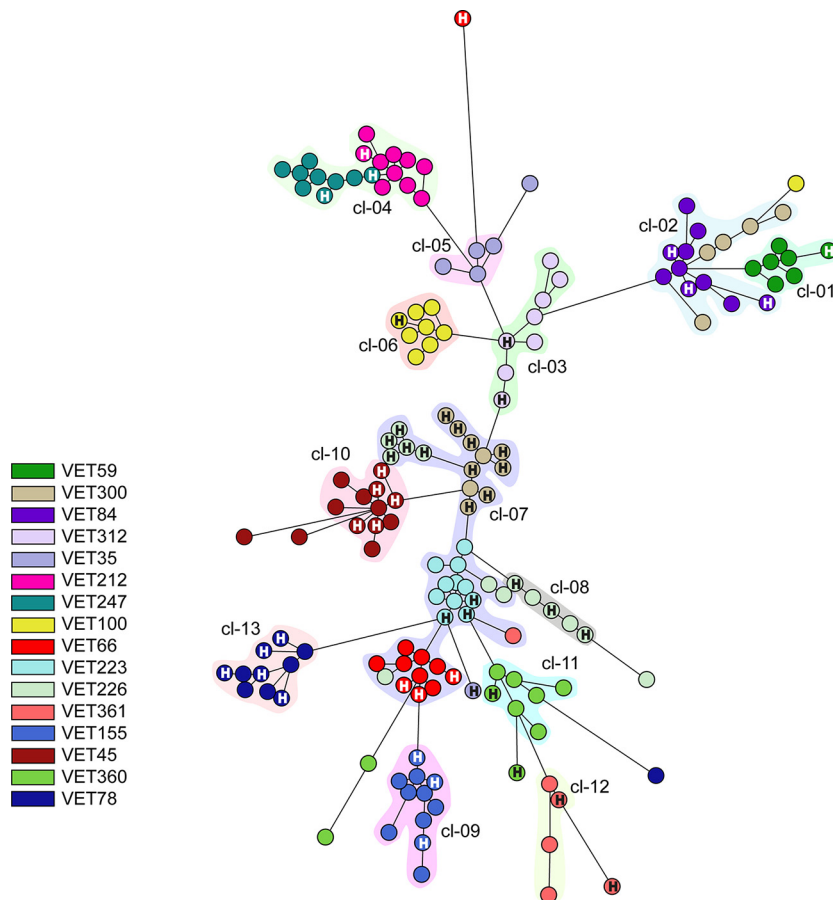
FIG 2 Minimum spanning tree displaying transmission of LA-MRSA between veterinarians and household members. Each node in this minimum spanning tree represents the WGM of a single LA-MRSA isolate. Nodes with identical colors represent isolates from the same household. The isolates obtained from the household members of each veterinarian are indicated with "H." The halos represent groups, as defined for the minimum spanning tree of Fig. 1.

ity between the maps was 92.7%, and, following the definition, these isolates are therefore regarded as different strains. Multiple transmission events were observed within the household of VET226. Over time, two different LA-MRSA strains were isolated from the two household members and veterinarian at multiple sampling moments. The first strain, found in household member identifier 226.1 (HHM226.1) at $t = 2m$ (nose and throat) and $t = 6m$ (nose), was also present in both isolates obtained from HHM226.2. The WGMs of three isolates from VET226 ($t = 0m$, $t = 2m$, $t = 14m$, nose) were considered highly related to the maps of this LA-MRSA strain (similarities ranged from 96.2 to 97.0%). The second LA-MRSA strain of HHM226.1 was found at $t = 6m$ (throat), $t = 10m$ (nose), and $t = 14m$ (nose). This strain was also present among the LA-MRSA isolates of the veterinarian on sampling moments $t = 6m$ (nose) and $t = 10m$ (throat), while another isolate ($t = 6m$, throat) was highly related (similarity of 96.3%) to this cluster (Fig. 3).

Besides transmission, persistent carriage or reacquisition of the same LA-MRSA strain among household members was found in eight different households. Indistinguishable WGMs were found for LA-MRSA isolates obtained from household members at different sampling moments (Fig. 2). In six of the households, this occurred in a single contact of each household, and in the two remaining households, persistence of LA-MRSA was observed in

two household members (VET226, VET45). The period of apparent persistence of LA-MRSA in household members in this study ranged from 4 to 8 months (Table 1).

DISCUSSION

In this study, we used whole-genome mapping, which revealed a considerable degree of genetic variation of LA-MRSA isolated from veterinarians and their household members from different geographic sources. Furthermore, we showed that there was frequent transmission of LA-MRSA between veterinarians and their household members. In addition, we have shown that both veterinarians and their household members carried LA-MRSA strains for prolonged periods of time, with carriage lasting up to 14 months. This provides arguments that LA-MRSA is a successful human colonizer.

Price et al. already showed that there is considerable genetic diversity among LA-MRSA CC398 isolates using whole-genome sequence-based single nucleotide polymorphism (SNP) analysis. However, in their study, a broad selection of LA-MRSA isolates originating from different countries and various sources and isolated between 1993 and 2010 were used. In addition, only 30% ($n = 26$) of the LA-MRSA isolates in this study originated from humans. Genetic diversity is to be expected in such a broad selection of LA-MRSA strains. In our study, all LA-MRSA isolates orig-
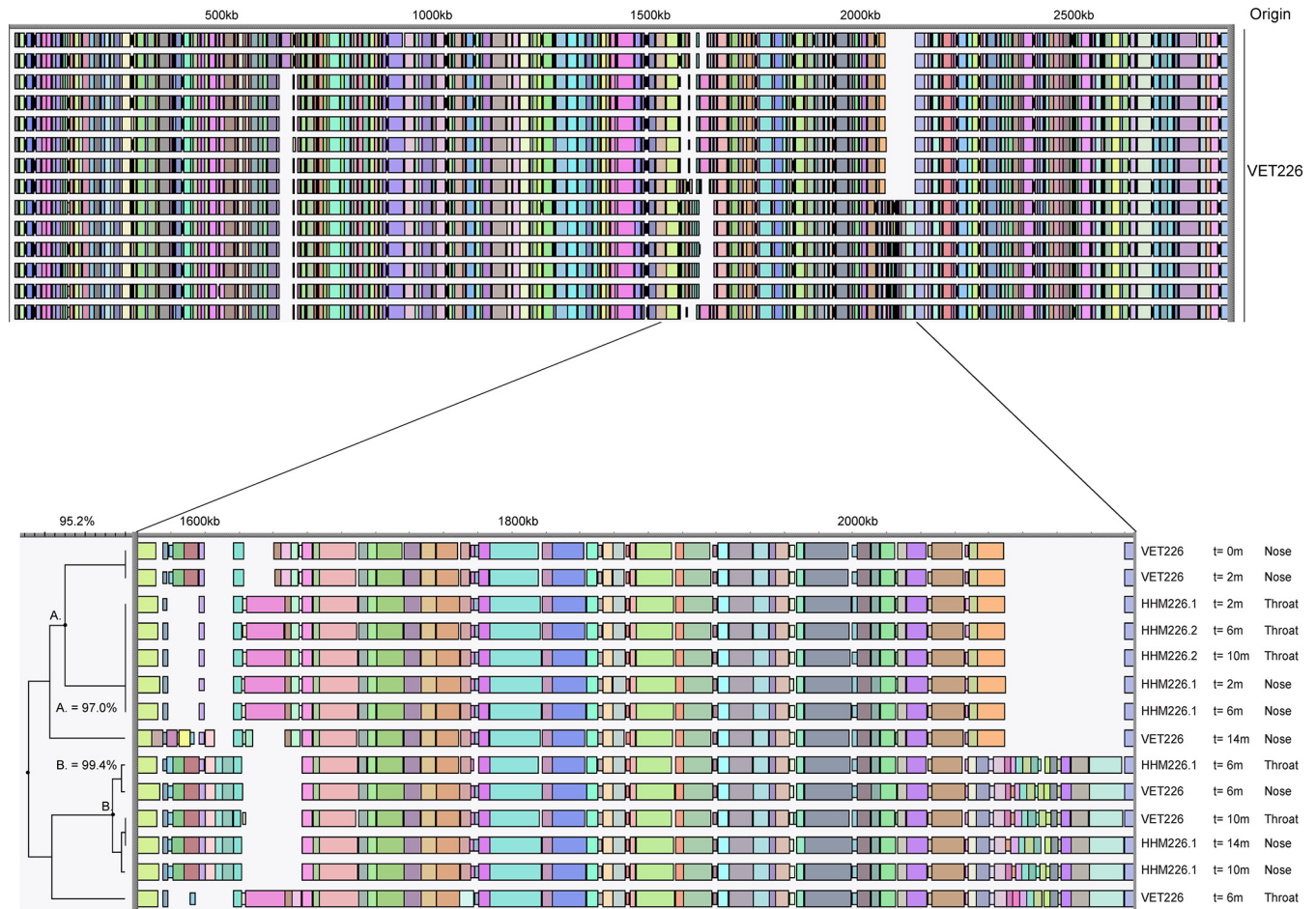
FIG 3 Whole-genome maps of isolates obtained from household (VET226) displaying colonization and transmission with different LA-MRSA strains at multiple sampling moments. The blocks in the maps represent the restriction fragments, and blocks that were considered to have the same size carry the same color. Blocks with reduced height were ignored in the comparison of the profiles. The upper panel of the figure displays the complete WGMs, and the lower panel depicts a zoomed-in region of the maps to display the variation in the restriction fragments. The origin of the sample, the sampling moment, and the anatomic location of the sampling is indicated on the right. The dendrogram on the left displays two clusters, A and B, and the similarity between the most distant members of the clusters.

inated from the Netherlands, were cultured from humans (specifically veterinarians and their household members), and were isolated within a period of 14 months. Even within this narrow selection, we still observed a considerable degree of genetic diversity. However, even with a high-resolution typing method such as whole-genome mapping, LA-MRSA remains a clade that is genetically more homogenous than other MRSA variants. This was illustrated by the fact that WGMs of the most distinct LA-MRSA isolates used in this study still had an 83.9% similarity, whereas WGMs of other MRSA variants yielded similarities of approximately 60 to 70%. For example, within a selection of seven pairs of MRSA isolates that all yielded MC8 and *spa* type t008, the most distinct maps showed a similarity of 74% (data not shown). In a minority of cases, we were unable to make a clear distinction between isolates obtained from two different veterinarians. This indicates acquisition of the same LA-MRSA strain in both veterinarians. A possible explanation may be that these veterinarians were colonized with LA-MRSA while visiting pig farms. Considering that pigs are distributed from one source to several farms, it is likely that identical strains are present in different farms (19, 20).

However, this remains speculation, as we are unaware of what type of livestock farms the veterinarians visited and what the frequency of these visits were.

Veterinarians and their household members were sampled longitudinally. Analysis of the WGMs of the LA-MRSA isolated during this period showed they carried the same strain up to 14 months. Whether this reflects persistent carriage or reacquisition of the same LA-MRSA strain remains uncertain, but the veterinarians in this study generally visited two to three different farms each working day and up to 10 different farms each week. Moreover, the household members had no direct contact with livestock animals. Therefore, it is likely that this reflects persistence of LA-MRSA within one individual and not reacquisition. Also, Köck et al. showed that 59% of the subjected farmers enrolled in their study did not clear MRSA colonization during leave, corroborating our hypothesis that farmers are more likely to be persistently colonized with LA-MRSA than transiently contaminated (21).

Since its emergence in 2003, the ability of LA-MRSA to cause transmission from humans to humans has been a subject of debate. Many of the reports describe the limited transmissibility of

the LA-MRSA (CC398) clade in general and rely on suboptimal typing methods, such as *spa* typing, making it difficult to determine whether these events were actual transmissions (14, 15, 22, 23). In our study, we focused on presumed LA-MRSA transmission events in a well-defined longitudinal cohort study using a high-resolution typing technique, and this revealed that the presumed transmissions were indeed likely transmissions in nearly every household. However, we do acknowledge that the previously described transmissibility studies were performed in hospital settings and that the outcome might differ considerably under these conditions. Contact between veterinarians and their household members will be more frequent and prolonged than contact between health care workers and patients and between patients. Therefore, transmission will be more likely to occur in households of veterinarians than in health care facilities. To assess whether the presumed nosocomial transmission of LA-MRSA is really occurring or whether this is a misinterpretation due to the use of low-resolution typing methods, we are currently analyzing isolates obtained from persons involved in presumed nosocomial transmission of LA-MRSA.

In conclusion, our results show that LA-MRSA is genetically diverse and that this genetic variation can be used to characterize LA-MRSA strains. Also, we showed that carriage of LA-MRSA in veterinarians and their household members can be persistent, lasting up to 14 months. Furthermore, this study demonstrates that transmission of LA-MRSA between veterinarians and their household members occurs, posing a potential risk for spread in the community of this highly resistant pathogen.

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