Dynamics and Determinants of *Staphylococcus aureus* Carriage in Livestock Veterinarians: A Prospective Cohort Study

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**Background.** Since 2003, a new clade of methicillin-resistant *Staphylococcus aureus* (MRSA) belonging to clonal complex (CC) 398 and associated with animal husbandry has emerged in the Netherlands. The purpose of this study was to determine the dynamics of carriage in persons with direct contact to livestock.

**Methods.** A 2-year prospective cohort study was performed in which the anterior nares and oropharynx of 137 livestock veterinarians were sampled for the presence of *S. aureus* every 4 months during the first year and again 1 year later. All *S. aureus* isolates were genotyped by staphylococcal protein A (*spa*) typing and with multilocus variable-number tandem repeat analysis (MLVA).

**Results.** The mean prevalence of MRSA CC398 carriage was 44% (range, 42%–46%), and for *S. aureus* the prevalence was 72% (range, 69%–75%). Thirty-two veterinarians (23%) were always carrying MRSA CC398 and 18 of those (56%, 13% of all veterinarians) had identical MLVA types at all sampling moments.

**Conclusions.** A high proportion of veterinarians had persistent MRSA CC398 carriage during the 2-year study period, indicating that this variant may colonize humans for prolonged periods. Furthermore, prevalence of *S. aureus* carriage was extremely high, indicating that MRSA CC398 is not replacing the susceptible strains, but comes on top of it.

**Keywords.** *Staphylococcus aureus*; epidemiology; dynamics; CC398; persistence.

Infections with methicillin-resistant *Staphylococcus aureus* (MRSA) are associated with increased morbidity, mortality, and healthcare costs [1, 2]. Traditionally, MRSA has been considered a hospital-associated pathogen [3, 4]. In the last 10–15 years, MRSA has expanded its territory to the community, causing severe infections in previously healthy persons worldwide [5]. Surveillance data of MRSA in the Netherlands show that MRSA prevalence upon hospital admission is still extremely low (0.1%) [6].

A new clade of MRSA associated with animal husbandry has emerged in the Netherlands since 2003 [7, 8]. This so-called livestock-associated MRSA belongs to multilocus sequence type clonal complex 398 (MRSA CC398) [9]. After its emergence, an active screening program was developed, and subsequently a strong increase in MRSA prevalence was observed in humans [10, 11]. By the end of 2008, 42% of all newly identified MRSA strains in humans belonged to this clade (www.rivm.nl/mrsa). The main risk groups for MRSA CC398 carriage are humans with professional exposure to pigs and veal calves [12, 13]. MRSA CC398 is rarely found outside of these risk groups [14].

*Staphylococcus aureus* nasal carriage has been extensively studied in patients and healthy individuals [15–17].
Cohort studies distinguish 3 carriage patterns among healthy individuals. Persistent carriage occurs in approximately 20% (range, 12%-30%) of the population; about 30% (range, 16%-70%) are intermittent carriers, and about 50% (range, 16%-69%) are noncarriers [15, 18, 19]. Persistent carriers usually carry the same strain for extended periods of time, whereas intermittent carriers tend to host different strains over time [17, 19]. The underlying mechanisms are largely unknown.

Veterinarians who regularly work with pigs and veal calves are daily exposed to extreme high loads of methicillin-susceptible S. aureus (MSSA) and MRSA CC398. Therefore, the dynamics of S. aureus carriage in these persons might be different due to competition for the binding site in the anterior nares between MSSA and MRSA strains [20]. The purpose of this prospective cohort study was to elucidate the dynamics of S. aureus carriage and its determinants in Dutch livestock veterinarians.

MATERIALS AND METHODS

Study Design and Setting

A 2-year prospective cohort study was conducted in Dutch veterinarians who mainly work with pigs and veal calves. Veterinarians were recruited from April until December 2008 and were followed for 2 years after enrollment. Data were collected at baseline (0 months), and at 4, 8, 12, and 24 months after inclusion. This study was approved by the medical ethics committee of the St Elisabeth Hospital in Tilburg, the Netherlands (protocol number 0749).

Study Population

In April 2008, all veterinarians associated with the Pig Health Department (approximately 95% of Dutch swine veterinarians) of the Royal Dutch Veterinary Society were invited by mail to participate in the study and asked to complete a questionnaire to determine the eligibility for the study. Veterinarians were eligible for participation if they (1) were aged between 18 and 65 years, (2) had 1 or more household members who were willing to participate in another study in which the transmission of MRSA CC398 between veterinarians and household members was studied, (3) had professional contact with pigs or veal calves at least once every 2 weeks in the previous year, (4) did not live on a farm with pigs or veal calves, (5) had no household members with professional contact with pigs or veal calves, (6) had not been treated for colonization with MRSA in the previous 3 months, and (7) had provided written informed consent.

During a home visit, cultures were taken from the anterior nares and the oropharynx (Supplementary Figure 1). Additional data were collected using a questionnaire that comprised information on age, sex, smoking, composition of the household, exposure to livestock, antibiotic treatment 4 months prior to sampling, and infections. Veterinarians were asked to take nasal and oropharyngeal cultures in the morning before visiting the stables and additionally to complete a short questionnaire on the presence of active infections and antibiotic usage at 4, 8, 12, and 24 months. Appropriate transport material with Amies medium (Transwab, Medical Wire & Equipment), instructions for sampling, and questionnaires were provided during the baseline home visit.

Microbiological Procedures

Nasal and oropharyngeal samples were directly plated on chromID S. aureus and chromID MRSA agar plates (bioMérieux, La Balme, France), and subsequently placed in a Mueller-Hinton (MH) broth supplemented with 6.5% sodium chloride. The overnight MH broth was subcultured onto both chromID S. aureus and chromID MRSA agar plates. All agar plates were read after 18–24 hours of incubation at 35°C–37°C according to the manufacturer’s instructions [21]. All cefoxitin-resistant isolates were tested using polymerase chain reaction for the presence of the mecA and nuc genes [22, 23]. All S. aureus isolates were genotyped by staphylococcal protein A (spa) typing [24] and multilocus variable number of tandem repeat analysis (MLVA) [25] and were stored at –80°C in the Microbank (Pro-Lab Diagnostics) preservation system.

Definitions of the Carrier State

Determination of the MRSA and MSSA carrier state was based on the 5 sampling moments during the 2-year study period (0, 4, 8, 12, and 24 months after inclusion). For each sampling moment, veterinarians were considered MRSA or MSSA positive when either the nasal or oropharyngeal swab harbored MRSA or MSSA. Veterinarians who were MRSA or MSSA positive at all 5 sampling moments were considered persistent carriers. In the case that one sampling moment was missing and the veterinarian was regarded as a persistent carrier as well. Veterinarians for whom only 1–4 samples yielded MRSA or MSSA were identified as intermittent carriers, and veterinarians who did not have any positive sample were considered noncarriers.

Statistical Analyses

All analyses were performed using SPSS software, version 19.0 for Windows (SPSS Inc, Chicago, Illinois). The mean prevalence was calculated by averaging the prevalences of all sampling moments. Differences in continuous variables between groups were tested with Student t test or Mann-Whitney U test when applicable, and differences in categorical variables between groups were tested with the Pearson $\chi^2$ test. The statistical tests were 2-tailed and $P \leq .05$ was considered statistically significant. All univariate analyses were performed in a generalized estimating equation model using a Poisson distribution with robust covariance estimators, to control for violation of
the distribution assumption that the variance equals the mean, and an independent correlation matrix for the multiple samples per veterinarian [26]. The carrier state of a given sampling moment was used as a possible predictor for the carrier state of the next sampling moment, corrected for the fact that multiple samples came from one person (clustered data). A sensitivity analysis was performed to establish the effect of missing samples on the carrier state.

RESULTS

Enrollment

In April 2008, 361 livestock veterinarians were asked to participate in the study. Two hundred twenty-five (62%) veterinarians responded, and 137 (61%) were eligible and included in the study (Figure 1).

Staphylococcus aureus carriage

During the 2-year study period, 137 veterinarians were screened for MRSA and MSSA in nares and oropharynx on 5 consecutive sampling moments. Among 1348 samples, 724 (54%) harbored S. aureus strains. Table 1 describes the carrier state and mean prevalence of MRSA, MSSA, and S. aureus. When broth enrichment would not have been used, 187 (26%) S. aureus–positive samples would have been missed. MRSA CC398 was recovered in 294 of 674 sampling moments (44%). There was a limited additional yield of oropharyngeal samples for the detection of MRSA CC398, as 17 (5.8%) samples originating from 9 different veterinarians were only positive in the oropharynx. Consequently, 6 veterinarians would have been defined as intermittent carriers instead of persistent carriers and 2 veterinarians would have been classified as noncarriers instead of intermittent carriers.

Throughout the study, 11 of 685 (1.6%) sampling moments were not received. Sensitivity analysis on the effect of missing samples had no relevant consequences on the conclusions (data not shown). A total of 133 (97%) veterinarians completed and returned the general questionnaire. The general characteristics and the frequencies of contact with livestock during the study period are shown in Table 2. Persistent and intermittent MRSA CC398 carriers reported significantly more intensive contact with pigs than veterinarians who did not carry MRSA CC398 (P = .016 and P = .001, respectively). The vast majority of veterinarians who carried MRSA CC398 or MSSA during the first year were still positive 1 year later for MRSA CC398 (31/39 [79%]; 1 sample missing) or MSSA (17/19 [89%]; 1 sample missing, respectively).

Results of spa Typing and MLVA

A total of 450 MRSA and 274 MSSA strains from nares and oropharynx were genotyped by spa typing and MLVA. Seventeen different spa types and 23 different MLVA types (MTs) were found among the 450 MRSA isolates; the majority of spa

Table 1. Mean Prevalence and Carrier State of Methicillin-Resistant Staphylococcus aureus (MRSA) CC398, MRSA Non-CC398, Methicillin-Susceptible S. aureus, and S. aureus Among 137 Livestock Veterinarians

<table>
<thead>
<tr>
<th>Organism</th>
<th>Mean Prevalence, % (Range)</th>
<th>Carriage Pattern, No. (%)</th>
<th>Carriage Pattern, No. (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MRSA CC398</td>
<td>43.6 (41.6–46.3)</td>
<td>Persistent, No. (%)</td>
<td>Intermittent, No. (%)</td>
</tr>
<tr>
<td>MRSA non-CC398</td>
<td>1.0 (0.7–1.5)</td>
<td>32 (23.4)</td>
<td>56 (40.9)</td>
</tr>
<tr>
<td>MSSA</td>
<td>27.2 (22.1–30.5)</td>
<td>18 (13.1)</td>
<td>57 (41.6)</td>
</tr>
<tr>
<td>S. aureus</td>
<td>71.7 (69.1–74.8)</td>
<td>65 (47.4)</td>
<td>57 (41.6)</td>
</tr>
</tbody>
</table>

Abbreviations: MRSA, methicillin-resistant Staphylococcus aureus; MSSA, methicillin-susceptible Staphylococcus aureus.

a Based on 5 consecutive sampling moments during the 2-year study period.

b A persistent carrier was a person with all nasal or oropharyngeal cultures positive for MRSA (5 sampling moments); noncarriers had no positive cultures; intermittent carriers were the remaining persons.

c Because MRSA and MSSA could coexist in one sample, the numbers do not add up.
We found a high prevalence of MRSA CC398 colonization (44%) in 137 veterinarians who work mainly with pigs and veal calves. In total, 88 veterinarians (65%) carried MRSA CC398 at least transiently. Others have also found high carriage rates of MRSA CC398 in veterinarians and livestock caregivers.

### Determinants for MRSA CC398 Carriage

The result of the baseline sample was highly predictive for subsequent findings. Thirty-one of 59 (52.5%) veterinarians with a baseline sample that contained MRSA CC398 were carrying MRSA CC398 in all 4 subsequent samples. These rates were significantly lower in veterinarians who harbored MSSA at baseline (3/40 [7.5%]; P < .001) and in veterinarians who did not carry *S. aureus* (3/37, [8.1%]; P < .001). In contrast, 46 of 77 (59.7%) veterinarians who did not carry MRSA CC398 at the baseline sample harbored no MRSA CC398 in the 4 subsequent samplings. Furthermore, veterinarians who carried MRSA CC398 in the baseline oropharyngeal sample had significantly more MRSA-positive test results in the next 4 sampling moments than those who did not carry MRSA CC398 in the baseline oropharyngeal sample, irrespective of nasal results (17/27 vs 20/109; RR, 3.4; 95% CI, 2.0–5.4).

Carriage of MSSA (but not MRSA) was not found to protect veterinarians from the acquisition of MRSA CC398 (RR 0.98; 95% CI, .56–1.71) as compared to veterinarians who carried no *S. aureus* (Table 3).

### DISCUSSION

We found a high prevalence of MRSA CC398 colonization (44%) in 137 veterinarians who work mainly with pigs and veal calves. In total, 88 veterinarians (65%) carried MRSA CC398 at least transiently. Others have also found high carriage

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**Table 2. Baseline and Study Characteristics of Livestock Veterinarians by Methicillin-Resistant *Staphylococcus aureus* CC398 Carrier**

<table>
<thead>
<tr>
<th>General Characteristic</th>
<th>Persistent MRSA CC398 Carrier (n = 32)</th>
<th>Intermittent MRSA CC398 Carrier (n = 55)</th>
<th>MRSA CC398 Noncarrier (n = 46)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, median (IQR)</td>
<td>47.0 (41.0–52.5)</td>
<td>50.0 (42.0–53.0)</td>
<td>46.0 (39.5–53.3)</td>
<td>n.s.</td>
</tr>
<tr>
<td>Male sex</td>
<td>31 (96.9)</td>
<td>51 (92.7)</td>
<td>41 (89.1)</td>
<td>n.s.</td>
</tr>
<tr>
<td>Smoking</td>
<td>4 (12.5)</td>
<td>14 (25.5)</td>
<td>5 (10.9)</td>
<td>n.s.</td>
</tr>
<tr>
<td>Contact with livestock&lt;sup&gt;b&lt;/sup&gt;</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>≥3 mo contact with pigs</td>
<td>30 (93.8)</td>
<td>48 (87.3)</td>
<td>29 (63.0)</td>
<td>&lt;.05&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>≥3 mo with veal calves</td>
<td>3 (9.4)</td>
<td>3 (5.5)</td>
<td>5 (10.9)</td>
<td>n.s.</td>
</tr>
<tr>
<td>≥3 mo contact with horses</td>
<td>4 (12.5)</td>
<td>15 (27.3)</td>
<td>9 (19.6)</td>
<td>n.s.</td>
</tr>
<tr>
<td>≥3 mo contact with poultry</td>
<td>1 (3.1)</td>
<td>1 (1.8)</td>
<td>1 (2.2)</td>
<td>n.s.</td>
</tr>
<tr>
<td>≥3 mo contact with other animals</td>
<td>15 (46.9)</td>
<td>31 (56.4)</td>
<td>29 (63.0)</td>
<td>n.s.</td>
</tr>
</tbody>
</table>

Household characteristics

| No. of household members, median (IQR) | 3.0 (2.3–4.0) | 3.0 (1.0–4.0) | 3.0 (1.0–4.0) | n.s.   |
| No. of companion animals<sup>d</sup>, median (IQR) | 1.0 (1.0–2.0) | 1.0 (0.0–2.0) | 1.0 (0.8–2.3) | n.s.   |

Data are presented as No. (%) unless otherwise specified. Statistically significant relationships are bolded. P ≤ .05 was considered statistically significant.

Abbreviations: IQR, interquartile range (p25–p75); MRSA, methicillin-resistant *Staphylococcus aureus*; n.s., not significant.

<sup>a</sup> General characteristics of 4 veterinarians were missing.

<sup>b</sup> Total cumulative duration of contact with livestock during the first year of the study period.

<sup>c</sup> Both persistent and intermittent MRSA CC398 carriers compared with MRSA CC398 noncarriers.

<sup>d</sup> Total amount of cats and dogs in the household.
rates of MRSA CC398 by humans with professional contact to pigs [7, 14, 27, 28].

The mean prevalence of MSSA among veterinarians per measurement was 27%, approximately equal to what is normally found. The combined MSSA and MRSA prevalence was 72%, which is extremely high compared to the general population [6, 15, 16, 18, 19, 29]. We conclude that MRSA CC398 carriage does not replace the susceptible strains and adds up to the total carriage rate of S. aureus. High rates of S. aureus carriage in healthy pig farmers have been described before by Aubry-Damon et al in 2004 [30]. The prevalence of S. aureus among pig farmers (44.6% [50/112]) was significantly higher compared to nonfarmers (24.1% [27/112]). Five of the 50 S. aureus isolates (10%) were methicillin resistant. The exact figures are difficult to compare with our findings as they did not use broth enrichment in their culture method, and thereby probably underestimated the S. aureus prevalence [21]. Nevertheless, people who work with pigs and veal calves have much higher carriage rates of S. aureus than those who do not. As shown in our study, this is partly determined by the level and duration of exposure. Persistent carriers reported more frequent contact with pigs. This was also found in a recent study among veal calf farmers [31]. In addition to the high levels of exposure during work, carriage of MRSA CC398 may also be determined by characteristics of the individual. The importance of host characteristics for the nasal carriage state is widely accepted and has been reviewed recently [32]. Thirty-two veterinarians (23%) had MRSA-positive test results throughout the entire study period, and 18 of those (56%, 13% of all veterinarians) had 5 identical MTs and can therefore be considered as actual persistent MRSA CC398 carriers. This prolonged carriage pattern was also found by others. A recent report demonstrated that the majority of pig farmers (59%) did not lose their MRSA CC398 carriage after the holidays [33]. Furthermore, when volunteers were actively colonized with MSSA CC398, they often carried it for prolonged periods [34]. Conversely, several studies stated that MRSA CC398 is not a good colonizer in humans. A recent study among field workers with short-term occupational exposure to pigs and veal calves suggested a high rate of transient contamination, without many persistent colonization [35]. Another study showed that MRSA prevalence among veal calf farmers was strongly reduced (58%) after absence of animal contact [31]. The reasons for these discordant findings are unclear. Different levels of exposure in stables and duration of exposition may have contributed.

Carriage of MSSA could theoretically offer a protective effect for the acquisition of MRSA CC398, as bacterial interference among S. aureus strains has been described before [15, 31], possibly through competition of the binding site [20]. However, veterinarians who carried MSSA at a specific moment did not have a lower risk to be MRSA CC398 positive in the next sampling moment compared to veterinarians who did not carry S. aureus at all. A possible explanation for the observed differences might be that veterinarians are exposed to extremely high amounts of MRSA CC398 [31, 36], which overrules the normal protective effect.

The MRSA non-CC398 prevalence among veterinarians was significantly higher compared to the general population (2/137 [1.5%] vs 7/6496 [0.1%]; RR, 13.5; 95% CI, 2.0–69.9; P = .014) [6]. However, a recent study also showed that a surprisingly high fraction of MRSA strains (7.3%) from veal calf farmers did not belong to CC398 [31]. The 2 veterinarians who carried MRSA

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**Table 3. Methicillin-Resistant Staphylococcus aureus CC398 and Methicillin-Susceptible S. aureus Carriage in Relation to the Result of the Next Sampling Moment Among 135 Livestock Veterinarians (n = 535 Sampling Moments)**

<table>
<thead>
<tr>
<th>Nasal Carriage on Current Sampling Moment*</th>
<th>No. of Observations</th>
<th>MRSA CC398 Positive, No. (%)</th>
<th>MRSA CC398 Negative, No. (%)</th>
<th>RR</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nasal positive</td>
<td>116</td>
<td>16 (14)</td>
<td>100 (86)</td>
<td>0.95</td>
<td>.53–1.70</td>
</tr>
<tr>
<td>Nasal negative</td>
<td>193</td>
<td>28 (15)</td>
<td>165 (85)</td>
<td>Ref</td>
<td></td>
</tr>
<tr>
<td>Oropharyngeal</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Oropharyngeal positive</td>
<td>78</td>
<td>8 (10)</td>
<td>70 (90)</td>
<td>0.68</td>
<td>.33–1.39</td>
</tr>
<tr>
<td>Oropharyngeal negative</td>
<td>325</td>
<td>49 (15)</td>
<td>276 (85)</td>
<td>Ref</td>
<td></td>
</tr>
<tr>
<td>Nasal and/or oropharyngeal</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nasal positive</td>
<td>151</td>
<td>30 (20)</td>
<td>121 (80)</td>
<td>0.98</td>
<td>.56–1.71</td>
</tr>
<tr>
<td>Nasal negative</td>
<td>155</td>
<td>24 (16)</td>
<td>131 (84)</td>
<td>Ref</td>
<td></td>
</tr>
</tbody>
</table>

Abbreviations: CI, confidence interval; MRSA, methicillin-resistant Staphylococcus aureus; MSSA, methicillin-susceptible Staphylococcus aureus; Ref, reference group; RR, relative risk.

* Only persons at risk for MRSA acquisition were considered, that is, MRSA negative on current sampling moment.
non-CC398 isolates did not have any known MRSA risk factors, such as visits to healthcare facilities. Nonetheless, one veterinarian reported frequent contact with companion animals during the study period. Different prevalence studies have found very diverse prevalences in small/companion animals [37, 38]. A recent study demonstrated that transmission of MRSA between companion animals and humans can occur [39].

Among the isolated MSSA strains from veterinarians, the CC398 lineage was the largest single clade representing 52 isolates (19%), which is significantly more prevalent than in MSSA originating from the general population [40]. A possible explanation is that MSSA CC398 is prevalent among pigs in Europe as well [41], and transmission to veterinarians can occur frequently due to direct contact with pigs. Moreover, spa type t571 was found in both MRSA and MSSA strains. Several studies demonstrated that this spa type has recently emerged in human blood cultures in diverse countries, indicating more virulent strain properties [40, 42, 43]. The present study also identified 3 MRSA spa type t571 isolates, showing that this specific subclone could indeed acquire the mecA gene. This could become a potential threat for the public health in the near future.

To the best of our knowledge, this investigation is the first large, long-term, prospective cohort study among livestock veterinarians. Furthermore, we have analyzed all samples for the presence of both MRSA and MSSA, and all isolates were genotyped by spa typing and MLVA. Thereby, we were able to evaluate the dynamics of S. aureus carriage among livestock veterinarians in detail.

There are several limitations to our study. First, we did not take any samples from the stables that had been visited by the participating veterinarians. In general, livestock veterinarians visit multiple pig and veal calf farms daily. Because it is very likely that multiple MRSA subclones are present in the stables, we could not determine the exact source and transmission routes. Second, we might have missed some MSSA strains in samples with predominant MRSA growth, due to the use of S. aureus–selective plates. This may have resulted in an underestimation of MSSA prevalence, although MRSA and overall S. aureus prevalences were not affected. Finally, study subjects were relatively healthy individuals who were exposed to an extremely high load of MRSA. Therefore, our data on development of disease in carriers are not representative for other populations.

In summary, we found a mean MRSA CC398 prevalence of 44% among livestock veterinarians. Furthermore, 23% of veterinarians were persistent MRSA CC398 carriers, and 56% of those veterinarians had always identical MTs and can therefore be considered as actual persistent MRSA CC398 carriers. This indicates that regular livestock contact can indeed lead to persistent colonization. Further research is required to elucidate the exact host factors and strain characteristics responsible for persistent MRSA CC398 carriage among individuals.

Supplementary Data

Supplementary materials are available at Clinical Infectious Diseases online (http://cid.oxfordjournals.org). Supplementary materials consist of data provided by the author that are published to benefit the reader. The posted materials are not copyrighted. The contents of all supplementary data are the sole responsibility of the authors. Questions or messages regarding errors should be addressed to the author.

Notes

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All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

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