Department of Infectious Diseases and Pathobiology

Institute of Veterinary Bacteriology, Vetsuisse Faculty University of Bern

Director Head of Institution: Prof. Dr. Joachim Frey

Scientific supervision was provided by

Dr. Gudrun Overesch

Longitudinal study on Methicillin-Resistant *Staphylococcus aureus* colonisation and transmission in Swiss pig farms

Inaugural Dissertation

to be awarded the Doctoral Degree of the

Vetsuisse Faculty University of Bern

submitted by

Patrick Daniel Bangerter

Veterinarian

from Lyss, BE

2015

Approved by the Vetsuisse Faculty as inaugural dissertation on proposal from

Prof. Dr. Joachim Frey

Bern,

Dean of the Vetsuisse Faculty

University of Bern

Table of Contents

Summary....4

Abstract....6

Introduction....7

Materials and methods....9

Results....13

Discussion....18

Conclusions....22

References....24

Tables....27

Figures....28

Acknowledgements....35

Curriculum Vitae....36

Vetsuisse Faculty University of Bern 2015

Patrick Daniel Bangerter

Institute of Veterinary Bacteriology, katharina.gerber@vetsuisse.unibe.ch

Longitudinal study on Methicillin-Resistant *Staphylococcus aureus* colonisation and transmission in Swiss pig farms

Summary

To date knowledge about the dynamics of Methicillin-Resistant *Staphylococcus aureus* (MRSA) in pigs lacks details on individual animal level. Only few longitudinal studies have been accomplished so far and different transmission routes for the spread of MRSA on farms have been proposed, but precise data on the behaviour of MRSA within a herd over time is lacking. The aim of our study was therefore to determine the colonisation status of MRSA in the individual pig throughout each production stage from birth to slaughtering, in order to gain a better understanding of the substantial factors for transmission. Two farrow-to-finish herds and two grow-to-finish herds were included in the study. 1.728 nasal swabs of 390 pigs and 592 environmental wipes were collected at 11 different time points.
Conspicuous in the tracking of MRSA status on the individual pig was the intermittent colonisation throughout the whole production cycle at all stages of age. Almost all pigs from a MRSA-positive herd had changed the MRSA status several times which implies strongly, that pigs are transiently rather than permanently colonised and might point to a repeated contamination in a MRSA-positive environment. Typing the MRSA isolates found will help to understand the underlying mechanisms for MRSA colonisation. We highly recommend the necessity to define a MRSA status on herd-level instead of individual pig-level when considering prevention measures against MRSA. Thereby, prohibition of further spread of MRSA for countries with moderate prevalence as in Switzerland by defining MRSA-positive and negative farms and promote the trade of pigs only within herds of the same status, seemed to be feasible. This will also be important for combating further dissemination of LA-MRSA into healthcare facilities and the community via humans with close contact to animals, i.e., farmers, veterinarians, and slaughterhouse workers.

Keywords: LA-MRSA, Transportation, Slaughterhouse, Public Health, Swine

Longitudinal study on Methicillin-Resistant *Staphylococcus aureus* colonisation and transmission in Swiss pig farms

Patrick Daniel Bangerter [a], Xaver Sidler [b], Vincent Perreten [a] and Gudrun Overesch [a*]

[a] Institute for Veterinary Bacteriology, Vetsuisse Faculty, University of Bern, Laenggassstrasse 122, CH-3012 Bern, Switzerland

[b] Department of Farm Animals, Division of Swine Medicine, Vetsuisse Faculty, University of Zurich, Winterthurerstrasse 260, CH-8057 Zurich, Switzerland

*Corresponding author at: Institute of Veterinary Bacteriology, Vetsuisse Faculty, University of Bern, Laenggassstrasse 122, CH-3012 Bern, Switzerland. tel.: +41 31 6312438; fax: +41 31 6312634.

e-mail address: gudrun.overesch@vetsuisse.unibe.ch (G. Overesch).

e-mail addresses

patrick.bangerter@vetsuisse.unibe.ch

xsidler@vetclinics.uzh.ch

vincent.perreten@vetsuisse.unibe.ch

gudrun.overesch@vetsuisse.unibe.ch

Abstract

To date knowledge about the dynamics of Methicillin-Resistant *Staphylococcus aureus* (MRSA) in pigs lacks details on individual animal level. Only few longitudinal studies have been accomplished so far and different transmission routes for the spread of MRSA on farms have been proposed, but precise data on the behaviour of MRSA within a herd over time is lacking. The aim of our study was therefore to determine the colonisation status of MRSA in the individual pig throughout each production stage from birth to slaughtering, in order to gain a better understanding of the substantial factors for transmission. Two farrow-to-finish herds and two grow-to-finish herds were included in the study. 1.728 nasal swabs of 390 pigs and 592 environmental wipes were collected at 11 different time points.

Conspicuous in the tracking of MRSA status on the individual pig was the intermittent colonisation throughout the whole production cycle at all stages of age. Almost all pigs from a MRSA-positive herd had changed the MRSA status several times which implies strongly, that pigs are transiently rather than permanently colonised and might point to a repeated contamination in a MRSA-positive environment. Typing the MRSA isolates found will help to understand the underlying mechanisms for MRSA colonisation. We highly recommend the necessity to define a MRSA status on herd-level instead of individual pig-level when considering prevention measures against MRSA. Thereby, prohibition of further spread of MRSA for countries with moderate prevalence as in Switzerland by defining MRSA-positive and negative farms and promote the trade of pigs only within herds of the same status, seemed to be feasible. This will also be important for combating further dissemination of LA-MRSA into healthcare facilities and the community via humans with close contact to animals, i.e., farmers, veterinarians, and slaughterhouse workers.

Keywords: LA-MRSA, Transportation, Slaughterhouse, Public Health, Swine

1. Introduction

Since the 1990s, prevalence of methicillin-resistant *Staphylococcus aureus* (MRSA) was increasing in hospitals and the so called hospital-associated MRSA (HA-MRSA) has become a severe threat for human health (Enright et al., 2002; Grundmann et al., 2006). Few years later, cases of MRSA were detected in people without healthcare associated risk factors and new strains coming up called community-acquired MRSA (CA-MRSA) (Udo et al., 1993; Centers for Disease Control and Prevention (CDC), 1999; Chambers, 2001; Okuma et al., 2002; Kluytmans-Vandenbergh and Kluytmans, 2006). Ten years ago, a distinct multilocus sequence type ST398 of MRSA was first described in pigs and other livestock and was therefore referred to as livestock-associated MRSA (LA-MRSA) (Voss et al., 2005). Since then, numerous other countries have detected LA-MRSA in pigs and other animals (Khanna et al., 2008; Graveland et al., 2010; Mulders et al., 2010; Broens et al., 2011a) and research groups worldwide have reported on this pathogen. In 2009, an official monitoring on MRSA in pigs at slaughterhouses was launched in Switzerland. The prevalence of MRSA in 2009 was very low, at 2% (95% CI 0.9-3.9). The prevalence increased in the following year to 5.9% (95% CI 3.8-8.7) (Overesch et al., 2011) and remained stable in 2011 at 5.6% (95% CI 3.6-8.4) (Overesch et al., 2012). In 2012, a significant increase to 18.1% (95% CI 14.7-22.2) was recorded (Büttner et al., 2013), followed by an increase to 20.8% (95%CI 16.7-25.45) in 2013 (Büttner et al., 2014).

Colonised pigs might act as a MRSA reservoir for livestock but also for humans with close contact to animals, i.e., farmers and veterinarians. This situation focused special attention on LA-MRSA as a public health concern since MRSA ST398 has emerged as an important cause of MRSA infection and colonisation in humans at risk in some European countries (Lewis et al., 2008; van Rijen et al., 2008). It is important to be aware of the potential risk of MRSA transmission from animals to humans with close contact to pigs, even if rarely happened. Prevention of MRSA colonisation of pigs will help to reduce the spread of MRSA and also the risk for humans, to introduce MRSA in to health care and community.

Abundant studies have accomplished focusing on different risk factors for the spread and persistence of MRSA and the routes of transmission. Such factors appear to be, e.g., direct contact between pigs

and the environment as a source for MRSA (Broens et al., 2012; Friese et al., 2012). Herd size (Broens et al., 2011a) and production type, e.g., farrow-, wean- and grow-to-finish farms (Alt et al., 2011) are also determinants identified as risk factors correlated with the occurrence of MRSA in a herd. Higher prevalence of MRSA in pigs at slaughterhouse in contrast to the prevalence on farms point to a possible MRSA transmission at slaughterhouse (de Neeling et al., 2007) or during transportation from farm to the abattoir (Broens et al., 2011b). The increase in MRSA positive pigs from 0% to 60% during transportation (Broens et al., 2011b) indicates very rapid transmission and it shows clearly that management on farm, at transport and at slaughterhouse plays an essential role in the dissemination of MRSA. To date only little is known about the dynamics of MRSA in pigs because merely few longitudinal studies have been accomplished so far. Different studies found significant association between the colonisation status of sows and their piglets (Weese et al., 2011; Broens et al., 2012; Verhegghe et al., 2013) and Weese et al. (2011) suggested that colonisation could be age related.
An age related association was also observed by Burns et al. (2014). The latter study investigated the effect of *S. aureus* colonisation status of a sow on the colonisation status of her piglets and indicated that the status has an effect on the piglets. They have demonstrated that the proportion of *S. aureus* is different in certain production stage. Thus, the average carriage rate was at its highest two days after farrowing, followed by a decrease prior to weaning. Moreover it was shown, that the majority of pigs changed carriage status at least twice during their study and suggested, that piglets are normally transiently rather than permanently colonised from birth.
Other longitudinal studies examined the MRSA status mostly until slaughter age of the pigs (Broens et al., 2012; Verhegghe et al., 2013; Burns et al., 2014) or even just until the age of 70 days (Weese et al., 2011). The observations of Burns et al. (2014) based on an unusual clonal complex (CC9) and it is unclear whether it is possible to implement the results on the most prevalent European CC398.
Moreover other studies examined only one farm (Weese et al., 2011; Burns et al., 2014) and it is doubtful whether the results are generally applicable.
However, there are no precise studies which observed the MRSA status in the individual pig throughout each production stage up to and including transport and slaughtering. The aim of our study was therefore to determine the individual colonisation status of MRSA in order to gain a better understanding of the substantial factors considering the prevention of the spread of MRSA and to

identify targets for possible intervention measures to prevent further spread of MRSA in Swiss slaughter pigs.

2. Materials und methods

2.1. Farm characteristics and animals

Pigs from four Swiss pig farms, two farrow-to-finish farms (ff-I, ff-II) and two grow-to-finish farms (gf-I, gf-II) were recurrently tested for the presence of MRSA between Mai and December 2014 during one production cycle, as well as additional pigs on transportation from three farms.

Farm ff-I was a closed farrow-to-finish pig farm which operates in a continuous flow manner. No other livestock was present. The farm consisted of 75 sows, 50 remount animals, two farrowing rooms for 18 sows in each room, one weaner accommodation and a finishing unit with a capacity for 200 fattening pigs. The farm had a three-week batch monitoring system. The MRSA status of the farm was determined as positive in a previous screening. One cohort of ten pregnant sows approaching delivery was selected for starting sampling and placed in one farrowing room. Four sows were placed in pens with possible direct contact to the neighbour pen through an open fence. The other six sows had no contact. Samples and time points are listed in Table 1. In addition, two boars present at the farm were sampled as well.

Farm gf-I was a grow-to-finish pig farm which operated in a continuous flow manner and purchased the grower pigs from farm ff-I but also from other breeders. The farm ran a calf fattening unit, additionally. The farm had seven finishing pens with a capacity for 280 fattening pigs. The MRSA status of the farm was determined as positive in a previous screening. One finishing pen containing 37 fattening pigs, grown on farm ff-I was selected for sampling. Samples and time points are listed in Table 1.

Farm ff-II was a farrow-to-finish pig farm which operated in a continuous flow manner. No other livestock was present. The farm consisted of 42 sows, one farrowing room with 12 farrowing pens, one weaner accommodation and a finishing unit with a capacity for 250 fattening pigs. Remount

animals were purchased. The farm had no regularly batch monitoring system. The MRSA status of the farm was determined as negative in a previous screening. One cohort of three pregnant sows approaching delivery was selected for starting sampling. All three sows were placed in pens with possible direct contact to the neighbour pen through an open fence. 5 other pens were also occupied. The status of those sows was unknown. Samples and time points are listed in Table 1. In addition, one boar present at the farm was sampled as well.

Farm gf-II was a grow-to-finish pig farm which operated in an all in/all out flow manner. The farm ran a dairy cow unit, additionally. The farm had one finishing pen with a capacity for about 90 fattening pigs. The MRSA status of the farm was determined as positive in a previous screening. A total of 87 fattening pigs purchased from one breeder were selected for sampling. Samples and time points are listed in Table 1.

2.2. Collection of samples

Nasal swabs from individual pigs were taken at different time points during a production cycle up to and including slaughtering. At each time point nasal swabs and when indicated additional environmental wipes were taken (table 1). At the two farrow-to-finish farms (ff-I, ff-II), sows were sampled three times and their offspring nine times. Fattening pigs at the two grow-to-finish farms (gf-I, gf-II) were sampled five times. Nasal samples were collected using transport swabs (Transwab® Amies MW172, England and Uni-Ter Amies CLR, Meus S.r.l., Italy) from both nares from the pigs. Environmental wipes (lairs, wall, watering place, manger and steel parts) were collected from the farrowing pens, the weaner accommodation, the finishing units and the lairages at slaughterhouse as well as from the lorrie, using wipes (Triko-Tex®, Chicopee Europe, 18cm x 32 cm) moistened with distilled water. Each wipe was individually placed in a sterile stomacher bag.

At the upcoming time points, following samples were obtained. Time points 1 to 6 were only applicable on farrow-to-finish farms (ff-I, ff-II). One week prior to farrowing at time point 1, nasal samples from the sows after penning into the farrowing room were taken. Additional environmental wipes from the not yet used and cleaned farrowing pens were taken. At time point 2 within 24 hours after farrowing, nasal samples from the sows, their offspring and boars present on farm were taken. At

time point 3 with around ten days, only piglets were tested. Piglets were weaned at time point 4 with approximately four to five weeks of age and were merged into new groups. Each group was housed in separate pens within one room at the weaner accommodation. Nasal samples were taken from weaned piglets and sows. Additional environmental wipes from farrowing pens after holding pigs and from the not yet used and cleaned weaner accommodation were taken. At time point 5, two days after weaning, the same farrowing pens were tested again after cleaning. At time point 6, approximately six weeks of age, weaned piglets were tested. At the age of nine to ten weeks at time point 7, grower pigs on the two farrow-to-finish farms (ff-I, ff-II) were merged into new groups and moved to the first stage finishing. Nasal samples were taken as well as environmental wipes from weaner accommodation after holding pigs and from the not yet used and cleaned pens at the finishing unit. On the two grow-to-finish farms (gf-I, gf-II) grower pigs were purchased and penned in the finishing pens when sampling started. At time point 7, nasal samples were taken as well as environmental wipes from the not yet used and cleaned finishing pens. In addition environmental wipes from the lorrie before and after transporting pigs from farm ff-I to farm gf-I were taken. At time point 8, approximately 14 weeks of age, fattening pigs on the two farrow-to-finish farms (ff-I, ff-II) were moved to second stage finishing. Nasal samples were taken as well as environmental wipes from pens at first stage finishing after holding pigs and from the not yet used and cleaned pens at second stage finishing. On the two grow-to-finish farms (gf-I, gf-II) nasal samples were taken as well as environmental wipes from the used finishing pens (pigs were not moved). At the end of fattening, samples were taken at three different moments, i.e., before (tp9i) and after (tp9ii) transportation and after stunning or bleeding (tp9iii). Slaughter pigs from the four farms were transported to three different commercial abattoirs (slaughterhouse sh-I, sh-II and sh-III), namely farm ff-I and gf-I to slaughterhouse sh-I, farm ff-II to slaughterhouse sh-II and farm gf-II to slaughterhouse sh-III, respectively. At time point 9i at farm, nasal samples from all finishing pigs were taken, as well as environmental wipes from the not yet used lorrie and the used pens at the finishing units after loading the pigs. In addition environmental wipes from the not yet used lairages at slaughterhouse were taken as well. The lairages at slaughterhouse sh-II were already used that day and for that reason not cleaned. After penning at slaughterhouse at time point 9 ii, nasal samples from slaughter pigs were taken as well as environmental wipes from the lorrie

after transporting the pigs. At time point 9iii, nasal samples were taken just after stunning or bleeding the slaughter pigs and environmental wipes of lairages after holding pigs.

On transportation to the abattoir from farm ff-1, additional pigs (n=42) from one other farm were picked up by the same lorrie. First nasal samples from these pigs were taken on farm before transportation (tp9i). On transportation from farm gf-II, additional pigs from two other farms (farm 1, n=56; farm 2, n=21) were picked up also by the same lorrie. First nasal samples from these pigs were taken one day and two days before transportation (tp9i). Additional pigs from the other farms were located in separate lorrie section but contact between pigs was possible. Moreover on transportation of batch ff-I and batch gf-I, supplemental 10 pigs and 5 pigs, respectively, which were not part of the study were transported and tested as well but only at time point 9i, 9ii and 9iii.

Depending on movement or decease of pigs, the number of sampled pigs varied over time. Only results from pigs, which were consistently sampled during the complete period of the study, were given.

2.3. Sample processing for MRSA isolation

Samples were transported within three hours at ambient temperature to the laboratory. At farm ff-1 at time point 2, for reasons of different days of birth, the samples were taken at 5 different days. All samples were stored at 5 °C and were transported under cooled conditions (<12 °C) to the laboratory six days after the first samples were taken. After arrival, swabs were transferred into tubes containing 10 ml Mueller Hinton Broth supplemented with 6.5% NaCl. Each stomacher bag containing environmental wipes, 50 ml Mueller Hinton Broth supplemented with 6.5% NaCl was added and homogenised in a stomacher® 400 circulator (Seward Ltd., UK) during 15 s at 230 RPM. The samples were incubated aerobically at 37°C +/- 1°C for 24 h under agitation. One ml from each pre-enrichment was inoculated into 9 ml tryptone soy broth containing 3.5 mg/L cefoxitin and 75 mg/L aztreonam, and further incubated aerobically at 37°C +/- 1°C for 24 h. A loopful was then spread onto MRSA selective agar plates (BBL ™ CHROMagar ™ MRSA; Becton Dickinson, Franklin Lakes, NJ), which were incubated at 37°C +/- 1°C for 24 h. Pink to mauve-coloured colonies were regarded as suspicious and one presumptive colony was cultivated onto tryptone soy agar plates containing 5% sheep blood

(TSA-SB) (Oxoid Ltd, Basingstoke, England) at 37°C +/- 1°C for 24 h. *Staphylococcus aureus* was identified by matrix-assisted laser desorption/ionisation time-of-flight mass spectroscopy (MALDI TOF MS) (Biotyper 3.0, Bruker) using the direct transfer protocol recommended by the manufacturer. The identification of all MRSA isolates was confirmed by a PCR targeting the mecA gene encoding for methicillin-resistance (Stegger et al., 2011). Positive and negative control strains were included. The obtained MRSA isolates were subsequently stored at -80 °C in glycerol buffer for further analysis.

3. Results

In total, 1.728 nasal swabs of 390 pigs and 592 environmental wipes were collected on seven farms at 11 different time points. Individual results of animal and environmental samples are summarized in Fig. 1 to 6.

On **farm ff-I**, ten sows (A-J) sampled before farrowing at time point (tp) 1, three were MRSA-positive (sow C, D and F) (Fig. 1). Two of them were penned in farrowing pens which were previous to penning screened positive for MRSA (pen D and F). From the three MRSA-positive sows, two (sow C and F) had changed their MRSA status 24 hours after birth (tp2) to negative and stayed negative until piglets were weaned with approximately 28 days (tp4). Only two sows (sow B and J) were sampled negative at all three sampling moments. After tp4, sows have been omitted from the study and only their offspring was followed. Offspring of one MRSA-positive sow (sow C) was complete negative 24 hours after birth (tp2), whereas the majority of piglets from the two other positive sows were MRSA-positive as well. On the other hand, also offspring from the MRSA-negative sows were MRSA-positive 24 hours after birth (tp2). Twenty four hours after birth (tp2), 38.6% (44/114) piglets were MRSA-positive. After roughly ten days of life (tp3), the majority of the piglets were tested MRSA-positive, i.e., 84.2% (85/101).

Within the piglets, seven different MRSA colonisation-patterns to the different time points (tp2, 3 and 4) could be observed. The two most seen pattern were neg/pos/pos (n=32) and pos/pos/pos (n=28). The pattern pos/pos/pos was distributed among piglets with a frequency from zero (sow C) to 66.6% (sow H). Interestingly, sow C was MRSA-positive, but had no piglets with such pattern (0/10) and

sow H with the highest frequency (6/9; 66.6%) was tested MRSA-negative. On the other hand, the MRSA pattern neg/neg/neg had only been found ten times (10/95; 10.5%) in piglets from two sows, sow B which was tested MRSA-negative and sow C, tested MRSA-positive. The other observed MRSA patterns were the following; neg/pos/neg (n=13), pos/pos/neg (n=6), pos/neg/pos (n=4) and neg/neg/pos (n=2). After weaning, the environmental wipes of pens were almost all MRSA-positive, whereas wipes after cleaning the farrowing pens were all negative. For more details see figure 1.
With around 28 days (tp4), pigs (n=95) were merged in weaner groups K, L, M, N and O and penned at the weaner accommodation of which 76 pigs could be tested at time point 4, 6 and 7 consistently (Fig. 2 and Fig. 3). Thirty five of the weaned pigs which were sampled 3 times at the weaner accommodation (tp4, 6 and 7) stayed MRSA-positive at all tp sampled (35/81; 43.2%). Three pigs (6438, 6439 and 6442) were MRSA-negative throughout weaning and all other pigs (49/81; 60.5%) changed their MRSA status once or even twice. The prevalence at tp4, 6 and 7 was 73.7% (70/95), 82.0% (73/89) and 69.1% (56/81), respectively. Environmental wipes from the weaner accommodation were all MRSA-negative previous to penning, whereas after holding pigs nearly all environmental wipes form all pens were MRSA-positive. For more details see figures 2 and 3.
Thirty nine grower pigs out of three weaner groups (K, N and O) were merged together with approximately eight weeks (tp7) in two fattening groups (P and Q) (Fig. 2). Six weeks later (tp8), the two groups were rehoused in pens R and S. At the end of finishing, the MRSA status of individual pigs was intermittent during the entire fattening cycle. Six different MRSA colonisation-patterns to the different time points until the end of fattening (tp7, 8 and 9i) were observed. There were two patterns in which the individual pig was tested MRSA-positive two times (pos/neg/pos, n=4; pos/pos/neg, n=2), but this pattern was only seen in 6 pigs. On the other hand, there were three different patterns in which the individual pig was tested MRSA-positive only one time (pos/neg/neg, n=11; neg/neg/pos, n=10; neg/pos/neg, n=1), in total seen in 22 pigs. Seven pigs were tested three times negative. Such pigs had come from pen O at the weaner accommodation. The prevalence at tp8 and 9i was 7.9% (3/38) and 36.8% (14/38), respectively and therefore much lower than at earlier time points.
Environmental wipes from pens at the finishing unit (P, Q, R and S) were positive, previous to penning and after holding pigs as well (Fig. 2).

Twenty finisher pigs out of 38 were monitored and sampled until stunning. Within these slaughter pigs, six different MRSA colonisation-patterns to the different time points until stunning (tp9i, 9ii and 9iii) has been revealed. In general, the prevalence has increased towards slaughtering, i.e., from 7.9% (3/38) in the middle of the fattening period (tp8) to 36.8% (14/38) at the end of fattening (tp9i). Even after transportation (tp9ii) and after stunning (tp9iii) it had occurred that the individual MRSA status of a pig had changed. Seven pigs (7/20; 35.0%) which were tested MRSA-negative at tp9i and 9ii, remained negative even after stunning (tp9iii). Four pigs (4/20; 20%) had changed from MRSA-negative at tp9i and 9ii to positive after stunning (tp9iii). One pig was MRSA-positive at tp9i, changed to MRSA-negative at tp9ii but changed again to positive at tp9iii. In contrast, three pigs (3/20; 15.0%) had changed from MRSA-negative at tp9i to positive after transportation (tp9ii), but changed again to MRSA-negative after stunning (tp9iii). Another three pigs which were tested MRSA-positive at tp9i changed to negative after transportation (tp9ii) and stayed negative after stunning (tp9iii). Two pigs were tested MRSA-positive at tp9i, stayed MRSA-positive after transportation (tp9ii) but changed after stunning to MRSA-negative. Remaining three pigs were only tested twice and therefore not taken into consideration. For more details see figure 2. Supplemental ten pigs were finished and slaughtered together with the study pigs but only tested at the end of fattening at tp9 (Fig. 2). Only one pig was MRSA-positive at tp9i, but another four got positive at tp9iii and two had changed from MRSA-positive after transportation (tp9ii) to negative after stunning (tp9iii). Slaughter pigs (n=42) from one other farm were transported on the same lorrie to the abattoir and also tested at tp9 at the end of fattening (data not shown). In three of these animals, the intermittent MRSA status can also be seen, i.e., changing from MRSA-negative at tp9i and 9ii to positive after stunning (tp9iii). All other animals were sampled MRSA-negative. Environmental wipes taken from the lorrie previous to transportation were all MRSA-negative, whereas nearly all samples after transporting pigs were MRSA-positive. Environmental wipes taken from the unused lairages at slaughterhouse were all MRSA-negative (Fig. 2).

On **farm gf-I,** thirty seven grower pigs out of three weaner groups (K, L and M) from farm ff-I were purchased with approximately eight weeks and penned together for fattening (Fig. 3). Sixteen pigs could be tested until tp9iii, whereas another 21 pigs were only tested at tp7 and tp8, because they were slaughtered later. The MRSA status of individual pigs was intermittent during the entire fattening

cycle. In general the prevalence was decreasing, i.e., 50.0% (18/36) at tp8 and 25% (4/16) at tp9i, respectively. Four different MRSA colonisation-patterns of fattening pigs to the different time points (tp7, 8 and9i) were observed. There was one pattern seen in seven pigs in which the individual pig was tested MRSA-positive only at the beginning (pos/neg/neg). On the other hand, five pigs were tested MRSA-positive two times, but with different patterns (pos/pos/neg, n=4; pos/neg/pos, n=1). Three pigs were tested consistently MRSA-positive at all three time points. No pig was tested MRSA-negative three times. Environmental wipes from the finishing pen were MRSA-positive, previous to penning (tp7) as well as in the middle of the fattening cycle (tp8).

Even after transportation (tp9ii) and after stunning (tp9iii), MRSA status of individual pigs had changed. Fifteen finisher pigs out of 37 were monitored and sampled at tp9i, 9ii and 9iii until stunning. Seven pigs (7/15; 46.7%) which were tested MRSA-negative at tp9i and 9ii remained negative after stunning (tp9iii). Four pigs (4/15; 26.7%) has changed from MRSA-negative at tp9i and 9ii to MRSA-positive after stunning and three pigs (3/15; 20.0%) also changed to MRSA-positive after stunning but were also tested MRSA-positive at tp9i. One pig which was tested MRSA-positive at tp9i changed to negative after transportation (tp9ii) and stayed negative after stunning. For more details see figure 3. Supplemental five pigs were finished at farm gf-I and slaughtered together with the study pigs but only tested at the end of fattening at tp9. All pigs were MRSA-negative at tp9i and 9ii. Interestingly, two pigs got MRSA-positive at tp9iii after stunning. Environmental wipes taken from the unused lairages at slaughterhouse were all MRSA-negative (Fig. 3).

On **farm ff-II,** all three sows (sow A, B and C) sampled before farrowing at tp1 were MRSA-negative, as well as the environmental wipes from the farrowing pens before holding pigs (data not shown). None of the three sows changed their MRSA status until piglets were weaned (tp4) with approximately 35 days of age. After tp4, sows have been omitted from the study and only the offspring was followed. The offspring (n=39) was 24 hours after farrowing (tp2) completely negative and stayed negative until slaughtering. Also the environmental wipes from the farrowing pens after holding pigs were MRSA-negative (Fig. 4).

With around 35 days (tp4), pigs (n=30) were merged in one weaner group (D) and penned at the weaner accommodation. All weaned pigs stayed MRSA-negative at all tp sampled (tp4, 6 and 7)

throughout weaning. Environmental wipes from the weaner accommodation were all negative, both before and after holding pigs (Fig. 4).

Twenty eight grower pigs were merged together with approximately 11 weeks (tp7) in two fattening groups (E and F). Eight weeks later, the two groups were rehoused in pens G and H. Figure 4 illustrates the results of nasal swabs up to slaughtering (tp9). The MRSA status was consistently MRSA-negative until tp9ii, but changed to MRSA-positive after stunning (tp9iii) in all animals but one (2785). Environmental wipes from the pens at the finishing room were consistently MRSA-negative, both before and after holding pigs as well as the environmental wipes from the lorrie, previous and after transportation. In contrast, environmental wipes from the lairages at the slaughterhouse were MRSA-positive, before (2/15) as well as after (4/11) holding pigs (Fig. 4).

On **farm gf-II**, eighty seven grower pigs were merged together with approximately ten weeks (tp7) in one group and used for fattening (Fig. 5). The MRSA status of individual pigs was intermittent during the entire fattening cycle. In general the prevalence was decreasing at the beginning of fattening (tp7 and 8) but was slightly increasing towards the end of fattening (tp9i), i.e., 94.3% (82/87), 61.6% (53/86) and 77.0% (67/87), respectively. Six different MRSA colonisation-patterns over the different time points until the end of fattening (tp7, 8 and 9i) were observed. At the beginning of fattening (tp7), only five grower pigs were tested MRSA-negative (5/87; 5.7%). One grower pig stayed MRSA-negative (1/86; 1.2%) at all tp sampled (tp7, 8 and 9i) and another four had changed to MRSA-positive at tp8. There was one pattern in which the individual pig was tested MRSA-positive only one time (pos/neg/neg) seen in eight pigs. On the other hand, 35 pigs showed two different patterns in which the individual pig was tested MRSA-positive two times (pos/pos/neg, n=11; pos/neg/pos, n=24). Another 38 pigs were tested three times MRSA-positive (38/86; 44.2%). Environmental wipes from the finishing pen were MRSA-positive, previous to penning and after holding pigs as well (Fig. 5).

Also after transportation (tp9ii) and after stunning (tp9iii), the MRSA status of pigs had changed. Twenty five finisher pigs out of 87 were monitored and sampled until stunning. Within these slaughter pigs, five different MRSA colonisation-patterns to the different time points until stunning (tp9i, 9ii and 9iii) could be shown. Twelve pigs (12/22; 54.5%) were tested MRSA-negative after stunning (tp9iii), i.e., one was tested MRSA-negative at all tp sampled (tp9i, 9ii and 9iii), three were tested MRSA-positive at tp9i and changed to negative after transportation (tp9ii) and stayed negative after stunning

(tp9iii), three were only tested positive at tp9ii and five were tested MRSA-positive at tp9i and 9ii and changed after stunning (tp9iii) to MRSA-negative. Ten pigs (10/22; 45.5%) were tested MRSA-positive at all three time points (tp9i, 9ii and 9iii). Three pigs were only tested twice and therefore not taken into consideration. 5 pigs out of the 87 (5/87; 5.7%) grower pigs were MRSA positive at all five sampling moments throughout the entire fattening cycle. For more details see figure 5.
Figure 6 shows additional slaughter pigs from two other farms which were transported on the same lorrie to the abattoir together with the study pigs from farm gf-II. Sixty nine of the slaughter pigs were consistently tested 3 times. In the majority of the pigs, the intermittent MRSA status can also be seen. Within the pigs of these two farms, eight different MRSA colonisation-patterns to the different time points until stunning (tp9i, 9ii and 9iii) had been revealed. Twenty three pigs (23/69; 33.3%) were tested MRSA-positive at only one tp, i.e., one pig at tp9i, six pigs at tp9ii and 15 pigs (15/69; 21.7%) changed to MRSA-positive after stunning (tp9iii). Nine pigs were tested MRSA-positive at two tp, i.e., one pig at tp9i and 9ii, two pigs at tp9i and 9iii and six pigs at tp9ii and 9iii, respectively. Three pigs were tested MRSA-positive and 34 pigs were tested MRSA-negative at all three moments (tp9i, 9ii and 9iii) (Fig. 6). Environmental wipes taken from the lorrie previous to transportation were all MRSA-negative, whereas nearly one third of the samples after transporting pigs were MRSA-positive. Environmental wipes from the lairages at the slaughterhouse were MRSA-positive, before (1/11) as well as after (1/13) holding pigs (Fig. 5).

4. Discussion

The present study investigated the transmission of MRSA on individual pig level at four different pig herds. Tracking the MRSA status of the individual animal over time within a herd, from farrowing to slaughter including transport and the environment, provides a better understanding of the critical points which are potentially liable for both, spread and persistence of MRSA. We scrutinized the MRSA status of the individual pig during the whole production cycle. In our knowledge, this is the first study which tracks the status thorough up to the very end of the production.

Conspicuous in the tracking of the MRSA status in the individual was the intermittent colonisation, throughout the whole production cycle. The majority of all individual pigs from a MRSA-positive herd had changed the MRSA status at least two times (Fig. 1-3 and Fig. 5). Due to this circumstance, the prevalence was varying through the production cycle. In the MRSA-positive farrow-to-finish herd ff-I, there was a first peak in the prevalence at roughly ten days of life with 84.2% positivity. At weaning, the prevalence declined to 73.7% and was increasing again until six weeks of age to 82%. After that time point, the prevalence decreased consistently to less than 10% in the middle of the fattening period with 15 weeks of age. At the end of fattening, the MRSA prevalence was increasing again to 36.8% and the MRSA status was still changing in the individual, even after transportation or after stunning. The same pattern, but on a higher level was seen in the grow-to-finish herd gf-II, where the MRSA prevalence was on a peak of 94.3% at the beginning of fattening. In the middle of the fattening period, the prevalence was declined to 61.6% and was increasing again to 77.0% at the end of fattening. Varying prevalence in defined time periods during pig production was also demonstrated in various other studies (Daewaele et al., 2011; Weese et al., 2011; Burns et al., 2014). The intermittent MRSA colonisation in the individual, shown in the current survey, implies strongly that pigs are transiently rather than permanently colonised and that re- and decolonisation can occur at all stages of age, in piglets, in weaned pigs, in fattening pigs as well as in sows. Little is known about the mechanisms which are responsible for a transient or intermittent in contrast to a permanent colonisation. B. van Cleef et al. (2011) investigated the MRSA status in humans after short-term occupational exposure to pigs or veal calves. Their results indicated that short-term occupational exposure of humans on MRSA-positive farms frequently results in the acquisition of MRSA, but the majority of people who acquire MRSA lose the strain within 24 hours. Due to these results, they stated that the high prevalence of MRSA carriage in livestock farmers and veterinarians is partly the result of repeated contamination instead of real colonisation and it is uncertain, whether the detected MRSA on the nasal mucosa should be considered as a true colonisation or whether it is appropriate to describe it as contamination. This state could also be true for MRSA-positive pigs, as the intermittent colonisation might point to a repeated contamination in a MRSA-positive environment as described above. An option to confirm such hypothesis could be the isolation of MRSA-positive pigs from a

positive herd into a negative environment, and to determine, if and when they become MRSA-negative or not.

For *S. aureus* in humans, it is confirmed that persistent colonisation occurs only in 20% of persons, 60% are intermittent carriers, and 20% are non-carriers (Williams, 1963). The determinants of persistent and intermittent carriage and the mechanism leading to *S. aureus* nasal carriage in humans are multifactorial. Persistent carriers are frequently colonised by a single strain of *S. aureus* and the load is higher, whereas intermittent carriers may carry different strains over time (VandenBergh et al., 1999; Nouwen et al., 2004b; Nouwen et al., 2005; Eriksen et al., 2009). A cohort-study in which volunteers were artificially inoculated with a combination of *S. aureus* strains demonstrated that most persistent carriers selected their original resident *S. aureus* strain while non-carriers eliminated the Staphylococci from their nose. The researchers concluded that host characteristics seem to be responsible for the *S. aureus* carrier state and that an optimal balance between defence and attraction forces is critical for colonisation or elimination (Nouwen et al., 2004a). The characterisation of the MRSA isolates found in our study will show if only one or a few clones are involved in a MRSA-positive herd. Such condition would point to a farm-associated residential MRSA flora. Moreover, it would be interesting to compare this hypothesised farm-associated MRSA flora with the microbial flora of pigs on farm ff-II, which was throughout the complete study period MRSA-negative. These comparative analysis could point to differences in the composition of the mucosal flora witch are crucial for MRSA colonisation.

In human medicine, hospitals often give decolonisation therapy prior to surgery to patients who are colonised but there is no agreement about the indications for administration of topical intranasal treatment to patients, (Ayliffe et al., 1998; Herwaldt, 1999; Carter et al., 2002) since mupirocin administered intranasal to colonised patients often fails to eradicate MRSA or the effect of decolonisation is only short-term (Harbarth et al., 1999). Moreover, the emergence of mupirocin resistance emphasises the importance of the prudent use of this antibiotic as a major weapon in local therapy against MRSA and therefore, eradication strategies should be intended carefully (Cookson, 1998). Decolonisation therapy is therefore no alternative for pig production. Furthermore, decolonisation of single sows prior to parturition for instance is useless, when the farm is MRSA-positive. As shown in our study, MRSA-positive offspring occurs also from MRSA-negative sows.

Become MRSA-positive during transportation and/or after stunning could be pointed out as a critical important control point. The farm ff-II of which all pigs were tested MRSA-negative throughout the whole production cycle got MRSA-positive not until after stunning (Fig. 5). We also have demonstrated that pigs from another farm previous to transport tested MRSA-negative had become MRSA-positive after transportation on a lorrie with MRSA-positive pigs (Fig. 6). These findings are in agreement with Broens et al. (2011b) which have found that the prevalence of MRSA in pigs at abattoirs is higher than in pigs sampled on farms. They have demonstrated that pigs tested MRSA-negative at farm can become positive within hours during transportation. In one slaughter batch, which was MRSA-negative on arrival at slaughterhouse, 43.3% of pigs were tested positive after stunning. Broens et al. (2011) have discussed, that the most probable source of MRSA is the environment, since lairage at slaughterhouse had closed fencing which minimises contact with pigs from other batches. This indicates that lairages are a potential source of MRSA. Our findings support this theory, as environmental wipes taken from lairages at slaughterhouse sh-II were MRSA-positive, before and after MRSA-negative pigs were loaded. In consequence, all pigs but one (19/20) which were hold in this MRSA-positive lairage had changed their MRSA status from negative to positive after stunning in this slaughterhouse. In the same slaughterhouse, the probability of MRSA transmission from the environment to the pigs is further increased by the fact, that pigs are kept at least two hours at lairage before they get slaughtered. At least in this slaughterhouse improvement of hygiene measures to minimise the chance for pigs to become MRSA-positive is possible. To achieve a subsequent elimination and to avoid the transmission of MRSA from the lairages to slaughter pigs, a strict hygiene management at slaughterhouse is required. As our studies have shown this is effective since environmental wipes from other slaughterhouses were MRSA-negative.

Another likely source of MRSA for pigs are other pigs, either transported together in the same lorrie or get in contact at slaughterhouse. Our conclusions also support the results of de Neeling et al. (2007) which found surprising high prevalence of MRSA in healthy pigs at stunning and suggested that this could be due to exposure to MRSA at transportation and in lairages at abattoir. It might help to minimise possible MRSA transmission from pig to pig if contact between pigs on lorries or at slaughterhouse is strictly impossible, e.g., using completely closed fences. Another possibility could be to transport only MRSA-negative pigs instead of mixing pigs with unknown MRSA status.

Interestingly on farm ff-I, only sow B and sow C had piglets which were from farrowing until weaning (tp2, 3 and 4) MRSA-negative. These two litters were placed in pens with possible contact to each other but not to other MRSA-positive litters. This phenomenon proposes that separation of MRSA-negative pigs without contact to MRSA-positive pens could be a protective measure.
The necessity to define a MRSA status on herd-level instead of individual pig-level when considering prevention measures against MRSA was clearly shown in our study. The determination of the MRSA status of an individual pig reflects only a short moment in live. It is likely that the status could change immediately and therefore it is not possible for example to separate MRSA positive from MRSA negative pigs as a basis for subsequent eradication measures. In contrast, defining MRSA-positive and negative farms by screening individual pigs was shown to be very precisely in our study. Although prevalence changed over time, by testing pigs, either taking nasal swabs or collecting oral fluid (data not shown) and/or the environment, MRSA could always been detected if present on farm. Therefore, we conclude to define a farm either as MRSA-positive or negative and promote the trade of animals only within herds which have the same status. At that point it is essential for MRSA-negative farms to purchase animals only from breeders which are labelled as MRSA-free. This measure could help to prohibit further spread of MRSA within Swiss pig production. For eradication of MRSA from positive farms further knowledge of colonisation factors and microbial flora analysis are needed, helping to understand the mechanisms which lead to colonisation.
As clearly pointed out by our study, determination of the MRSA prevalence in pigs in Switzerland at slaughterhouse leads to an overestimation of the prevalence at farm level. As this is done up to now as part of the national MRSA antimicrobial resistance monitoring of livestock, authorities have to discuss whether it is necessary to take samples on farm level for a more precise prevalence, with a drawback of a much more elaborated sampling.

5. Conclusions

Conspicuous in the tracking of MRSA on individual pig level was the intermittent colonisation throughout the whole production cycle. This implies strongly that pigs are transiently rather than

permanently colonised and point to a repeated contamination, possible at all stages of age. Typing the MRSA isolates found as well as further studies on the microbial flora of MRSA-positive versus MRSA-negative pigs will elucidate possible discrepancies and promote understanding of the underlying mechanisms for MRSA colonisation. This will be crucial for eradication measures in the future.

We highly recommend the necessity to define a MRSA status on herd-level instead of individual pig-level when considering prevention measures against MRSA. As the prevalence in Swiss slaughter pigs is nowadays moderate, prohibition of further spread of MRSA within Swiss pig production by defining MRSA-positive and negative farms and promote the trade of animals only within herds of the same status, seemed to be feasible. This will also be important for combating further dissemination of LA-MRSA into healthcare facilities and the community via humans with close contact to animals, i.e., farmers, veterinarians, and slaughterhouse workers.

In view of the national MRSA antimicrobial resistance monitoring within livestock it has to discuss whether samples should be taken on farm level instead of at slaughterhouse for a more precise MRSA prevalence determination.

References

Alt, K., Fetsch, A., Schroeter, A., Guerra, B., Hammerl, J.A., Senkov, N., Geinets, A., Mueller-Graf, C., Braeunig, J., Kaesbohrer, A., Appel, B., Hensel, A., Tenhagen, B.A., 2011. Factors associated with the ocurrence of MRSA CC398 in herds of fattening pigs in Germany. BMC Vet. Res. 7.

Ayliffe, G.A.J., Buckles, A., Casewell, M.W., Cookson, B.D., Cox, R.A., French, G.L., Griffiths-Jones, A., Heathcock, H., Humphreys, H., Keane, C.T., Marples, R.R., Shanson, D.C., Slack, R., Tebbs, E., 1998. Revised guidelines for the control of methicillin-resistance *Staphylococcus aureus* infection in hospitals. JHI 39.

Broens, E.M., Espinosa-Gongora, C., Graat, E.A., Vendrig, N., van der Wolf, P.J., Guardabassi, L., Butaye, P., Nielsen, J.P., DE Jong, M.C., van de Giessen, A.W., 2012. Longitudinal study on transmission of MRSA CC398 within pig herds. BMC Vet.Res. 8, 58.

Broens, E.M., Graat, E.A., van der Wolf, P.J., van de Giessen, A.W., DE Jong, M.C., 2011a. Prevalence and risk factor analysis of livestock associated MRSA-positive pig herds in The Netherlands. Prev.Vet.Med. 102, 41-49.

Broens, E.M., Graat, E.A., van der Wolf, P.J., van de Giessen, A.W., DE Jong, M.C., 2011b. Transmission of methicillin resistant *Staphylococcus aureus* among pigs during transportation from farm to abattoir. Vet.J. 189, 302-305.

Burns, A., Shore, A.C., Brennan, G.I., Coleman, D.C., Egan, J., Fanning, S., Galligan, M.C., Gibbons, J.F., Gutierrez, M., Malhotra-Kumar, S., Markey, B.K., Sabirova, J.S., Wang, J., Leonard, F.C., 2014. A longitudinal study of *Staphylococcus aureus* colonization in pigs in Ireland. Vet. Microbiol. 174, 504-513.

Büttner, S., Flurina, S., Müntener, C., Jäggi, M., Overesch, G., 2013. Bericht über den Vertrieb von Antibiotika in der Veterinärmedizin und das Antibiotikaresistenzmonitoring bei Nutztieren in der Schweiz (ARCH-VET 2012).

Büttner, S., Mehmann, M., Müntener, C., Torriani, K., Overesch, G., 2014. Bericht über den Vertrieb von Antibiotika in der Veterinärmedizin und das Antibiotikaresistenzmonitoring bei Nutztieren in der Schweiz (ARCH-VET 2013).

Carter, A., Heffernan, H., Holland, D., Ikram, R., Morris, A., Roberts, A., Boyd, J., 2002. Guidelines fot the control of methicillin-resistant Staphylococcus aureus in New Zealand. Ministy og Health.

Centers for Disease Control and Prevention (CDC), C.f.D.C.a.P., 1999. Four pediatric deaths from community-acquired methicillin-resistant *Staphylococcus aureus* - Minnesota and North Dakota, 1997-1999. MMWR. Morbidity and mortality weekly report 48.

Chambers, H.F., 2001. The changing epidemiology of *Staphylococcus aureus*? Emerg. Infect. Dis. 7.

Cookson, B.D., 1998. The emergence of mupirocin resistance: a challenge to infection control and antibiotic prescribing practice. J. Antimicrob. Chemother. 41, 11-18.

Daewaele, I., Messens, W., De Man, I., Delputte, P., Herman, L., Butaye, P., Heyndrickx, M., Rasschaert, G., 2011. Sampling, prevalence and characterization og methicillin-resistant *Staphylococcus aureus* on two Belgian pig farms. Vet. Sci. Develop.

de Neeling, A.J., van den Broek, M.J.M., Spalburg, E.C., van Santen-Verheuvel, M.G., Dam-Deisz, W.D.C., Boshuizen, H.C., van de Giessen, A.W., van Duijkeren, E., Huijsdens, X.W., 2007. High prevalence of methicillin resistant *Staphylococcus aureus* in pigs. Vet.Microbiol. 122, 366-372.

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). PNAS 99.

Eriksen, N.H., Espersen, F., Rosdahl, V., Jensen, K., 2009. Carriage of *Staphylococcus aureus* among 104 health persons during a 19-month period. Epi. Infect. 115.

Friese, A., Schulz, J., Hoehle, L., Fetsch, A., Tenhagen, B.A., Hartung, J., Roesler, U., 2012. Occurrence of MRSA in air and housing environment of pig barns. Vet.Microbiol. 158, 129-135.

Graveland, H., Wagenaar, J.A., Heesterbeek, H., Mevius, D., van Duijkeren, E., Heederik, D., 2010. Methicillin resistant *Staphylococcus aureus* ST398 in veal calf farming: human MRSA carriage related with animal antimicrobial usage and farm hygiene. PLoS One 5, e10990.

Grundmann, H., Aires-de-Sousa, M., Boyce, J., Tiemersma, E., 2006. Emergence and resurgence of meticillin-resistant *Staphylococcus aureus* as a public-health threat. Lancet 368.

Harbarth, S., Dharan, S., Liassine, N., Herrault, P., Auckenthaler, R., Pittet, D., 1999. Randomized, placebo-controlled, double-blind trial to evaluate the efficacy of mupirocin for eradicating carriage of methicillin-resistant *Staphylococcus aureus*. Antimicrob. Agents. Chemother. 43, 5.

Herwaldt, L.A., 1999. Control of methicillin-resistant *Staphylococcus aureus* in the hospital setting. Am. J. Med. 106.

Khanna, T., Friendship, R., Dewey, C., Weese, J.S., 2008. Methicillin resistant *Staphylococcus aureus* colonization in pigs and pig farmers. Vet.Microbiol. 128, 298-303.

Kluytmans-Vandenbergh, M.F., Kluytmans, J.A., 2006. Community-acquired methicillin-resistant *Staphylococcus aureus*: current perspectives. Clin. Microbiol. Infect. 12 Suppl 1.

Lewis, H.C., Mølbak, K., Reese, C., Aarestrup, F.M., Selchau, M., Sørum, M., Skov, R.L., 2008. Pigs as source of methicillin-resistant *Staphylococcus aureus* CC398 infections in humans, Denmark. Emerg.Infect.Dis. 14, 1383-1389.

Mulders, M.N., Haenen, A.P., Geenen, P.L., Vesseur, P.C., Poldervaart, E.S., Bosch, T., Huijsdens, X.W., Hengeveld, P.D., Dam-Deisz, W.D., Graat, E.A., Mevius, D., Voss, A., van de Giessen, A.W., 2010. Prevalence of livestock-associated MRSA in broiler flocks and risk factors for slaughterhouse personnel in The Netherlands. Epidemiol.Infect. 138, 743-755.

Nouwen, J., Boelens, H., van Belkum, A., Verbrugh, H., 2004a. Human factor in *Staphylococcus aureus* nasal carriage. Infect. Immun. 72, 4.

Nouwen, J.L., Fieren, M.W., Snijders, S., Verbrugh, H.A., van Belkum, A., 2005. Persistent (not intermittent) nasal carriage of *Staphylococcus aureus* is the determinant of CPD-related infections. Kidney Int. 67, 9.

Nouwen, J.L., Ott, A., Kluytmans-Vandenbergh, M.F., Boelens, H.A., Hofman, A., van Belkum, A., Verbrugh, H.A., 2004b. Predicting the *Staphylococcus aureus* nasal carrier state: derivation and validation of a "culture rule". Clin. Infect. Dis. 39, 6.

Okuma, K., Iwakawa, K., Turnidge, J.D., Grubb, W.B., Bell, J.M., O'Brien, F.G., Coombs, G.W., Pearman, J.W., Tenover, F.C., Kapi, M., Tiensasitorn, C., Ito, T., Hiramatsu, K., 2002. Dissemination of new methicillin-resistant *Staphylococcus aureus* clones in the community. J. Clin. Microbiol. 40.

Overesch, G., Büttner, S., Perreten, V., 2012. Evolution of methicillin-resistance *Staphylococcus aureus* (MRSA). Fleischwirtschaft International.

Overesch, G., Büttner, S., Rossano, A., Perreten, V., 2011. The increase of methicillin-resistant *Staphylococcus aureus* (MRSA) and the presence of an unusual sequence type ST49 in slaughter pigs in Switzerland. BMC Vet.Res. 7, 30.

Stegger, M., Lindsay, J.A., Moodley, A., Skov, R., Broens, E.M., Guardabassi, L., 2011. Rapid PCR Detection of *Staphylococcus aureus* Clonal Complex 398 by Targeting the Restriction-Modification System Carrying *sau1-hsdS1*. J.Clin.Microbiol. 49, 732-734.

Udo, E.E., Pearman, J.W., Grubb, W.B., 1993. Genetic analysis of community isolates of methicillin-resistant *Staphylococcus aureus* in Western Australia. J. Hosp. Infect. 25.

Van Cleef, B., Graveland, H., Haenen, A., Van de Giessen, A., Heederik, D., Wagenaar, J., Kluytmans, J., 2011. Persistance of Livestock-Associated Methicillin-Resistant *Staphylococcus aureus* in Field Workers after Short-Term Occupation Exposure to Pigs and Veal Calves. J. Clin. Microbiol. 49.

van Rijen, M.M.L., van Keulen, P.H., Kluytmans, J.A., 2008. Increase in a Dutch Hospital of methicillin-resistant *Staphylococcus aureus* related to animal farming. Clin.Infect.Dis. 46, 261-263.

VandenBergh, M.F., Yzerman, E.P., van Belkum, A., Boelens, H.A., Sijmons, M., Verbrugh, H.A., 1999. Follow-up of *Staphylococcus aureus* nasal carriage after 8 years: redefining the persistent carrier state. J. Clin. Microbiol. 37, 8.

Verhegghe, M., Pletinckx, L.J., Crombe, F., Van Weyenberg, S., Haesebrouck, F., Butaye, P., Heyndrickx, M., Rasschaert, G., 2013. Cohort study for the presence of livestock-associated MRSA in piglets: effect of sow status at farrowing and determination of the piglet colonization age. Vet.Microbiol. 162, 679-686.

Voss, A., Loeffen, F., Bakker, J., Klaassen, C., Wulf, M., 2005. Methicillin-resistant *Staphylococcus aureus* in pig farming. Emerg.Infect.Dis. 11, 1965-1966.

Weese, J.S., Zwambag, A., Rosendal, T., Reid-Smith, R., Friendship, R., 2011. Longitudinal investigation of methicillin-resistant *Staphylococcus aureus* in piglets. Zoonoses and public health 58, 238-243.
Williams, R.E., 1963. Healthy carriage of *Staphylococcus aureus*: its prevalence and importance. Bacteriol. Rev. 27.

Table 1
Time points of sampling, compartment, age, age group, sample type and farm on which samples were taken.

Time point	Compartment	Age	Age group	Type of sample	Farm
1	Farrowing	1 week prior to farrowing	Sows	Nasal/Environment	ff-I, ff-II
2	Farrowing	within 24 hours post-natal	Sows/Piglets	Nasal	ff-I, ff-II
3	Farrowing	5-14 days	Piglets	Nasal	ff-I, ff-II
4	Farrowing/Weaning	24-38 days	Sows/Weaned p.	Nasal/Environment [a]	ff-I, ff-II
5	Farrowing			Environment	ff-I
6	Weaning	5-7 weeks	Weaned piglets	Nasal	ff-I, ff-II
7	Weaning/Fattening	8-11 weeks	Growers	Nasal/Environment [a]	ff-I, ff-II, gf-I, gf-II
8	Fattening/Finishing	15-19 weeks	Finishers	Nasal/Environment [a]	ff-I, ff-II, gf-I, gf-II
9 i	Finishing/Lorrie/Abattoir	21-25 weeks	Finishers	Nasal/Environment	ff-I, ff-II, gf-I, gf-II
9 ii	Lorrie/Abattoir	21-25 weeks	Finishers	Nasal/Environment	ff-I, ff-II, gf-I, gf-II
9 iii	Abattoir	21-25 weeks	Finishers	Nasal/Environment	ff-I, ff-II, gf-I, gf-II

[a] At rehousing the used and not yet used lairages were sampled.

Fig. 1. MRSA status of sows and offspring.
☐, MRSA-negative; ■, MRSA-positive; †, deceased; ➔, piglet moved;
ID, ear tag; tp, time point; n.t., not tested; 2/15, environmental samples (n positive/N total)

Fig. 2. Fattening pigs weaned and fattened on farm ff-I.
6164-6399, supplemental fattening pigs from farm ff-I which were not part of the study, sampled at tp 9 only.
☐, MRSA-negative; ■, MRSA-positive; †, deceased;
ID, ear tag; tp, time point; n.t., not tested; 2/15, environmental samples (n positive/N total)

Fig. 3. Fattening pigs weaned on farm ff-I and purchased for fattening to farm gf-I.
6232-6403, supplemental fattening pigs from farm gf-I which were purchased from the same breeder (ff-I) but not part of the study. Sampled at tp 9 only.
☐, MRSA-negative; ■, MRSA-positive;
ID, ear tag; tp, time point; n.t., not tested; 2/15, environmental samples (n positive/N total)

Fig. 4. Fattening pigs on farm ff-II.
☐, MRSA-negative; ■, MRSA-positive; †, deceased
ID, ear tag; tp, time point; n.t., not tested; 2/15, environmental samples (n positive/N total)

Fig. 5. Fattening pigs on farm gf-II.
☐, MRSA-negative; ■, MRSA-positive
ID, ear tag; No., number of pigs with the same result; tp, time point; n.t., not tested; 2/15, environmental samples (n positive/N total)

Fig. 6. Slaughter pigs of two other farms on the transport batch gf-II, sampled at tp 9 only.
☐, MRSA-negative; ■, MRSA-positive
ID, ear tag; No., number of pigs with the same result; tp, time point; n.t., not tested; n.e., no ear tag; 2/15, environmental samples (n positive/N total)

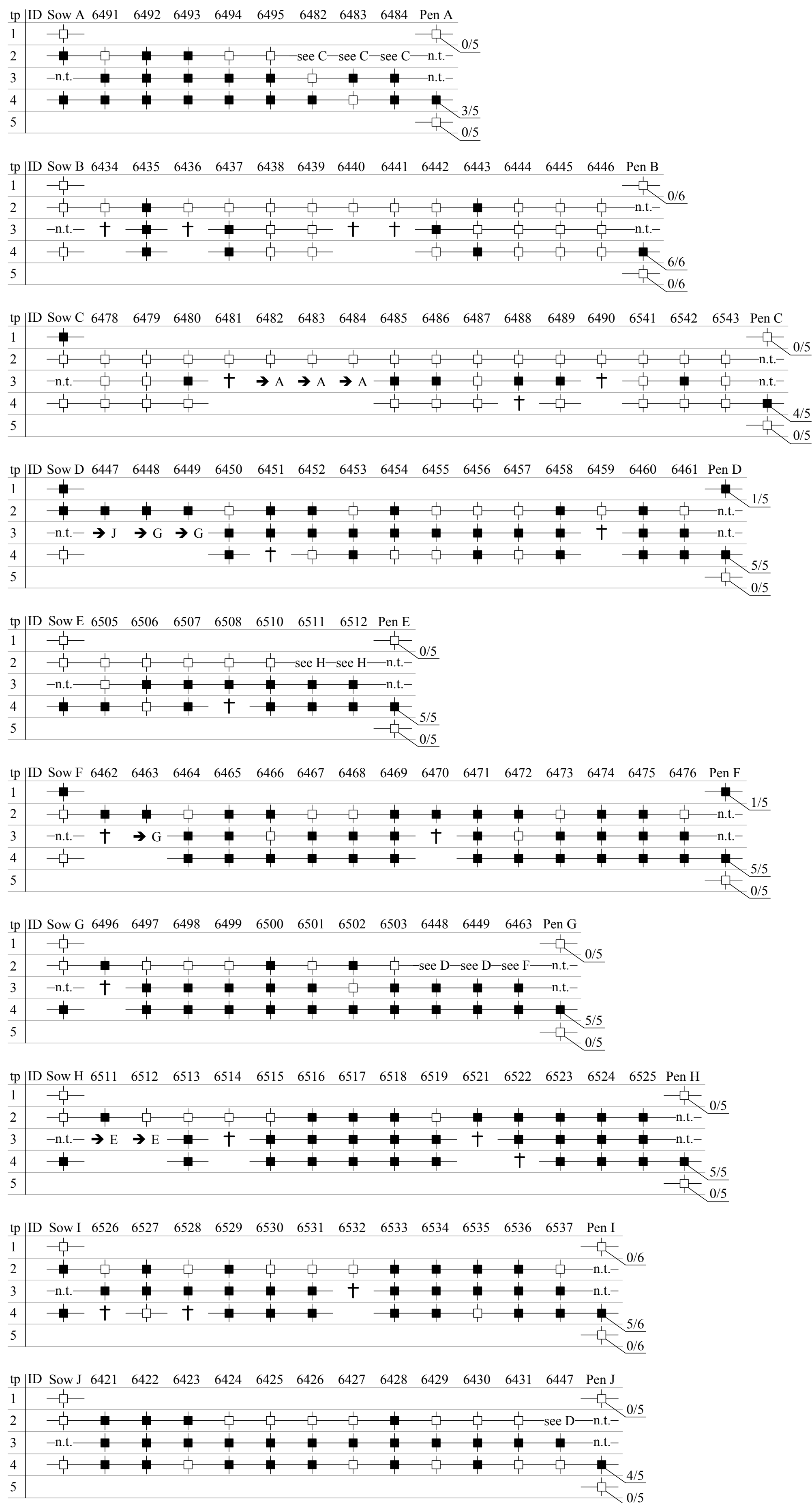
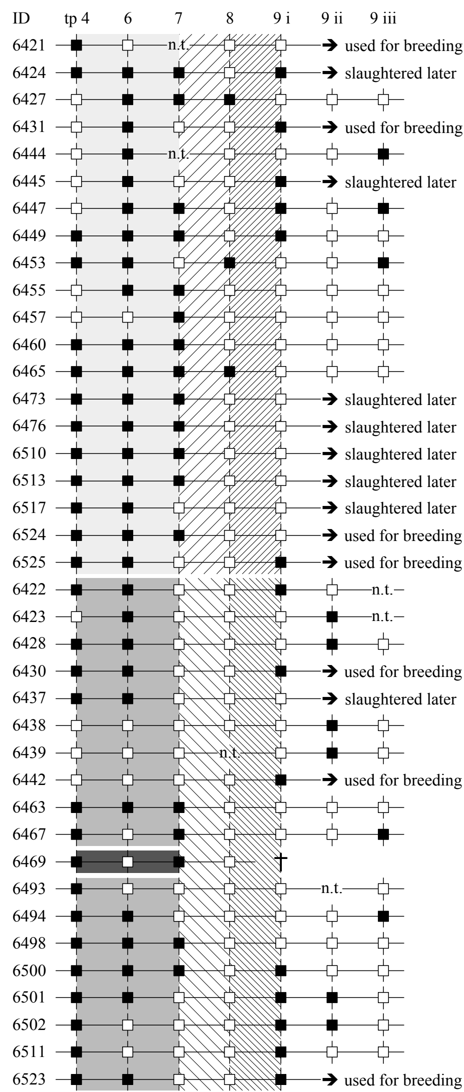
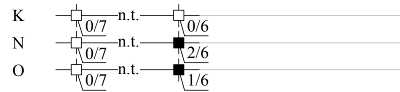


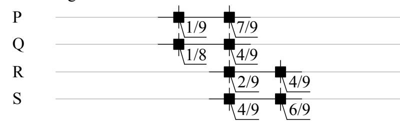
Fig. 1.



Weaner accommodation



Finishing unit



Lorry

Slaughterhouse

Supplemental pigs on transportation

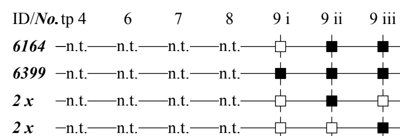
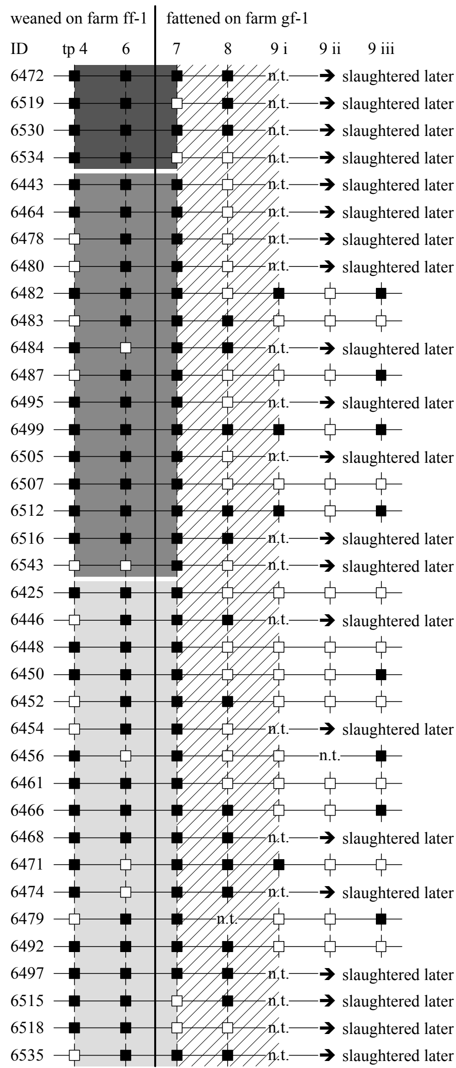
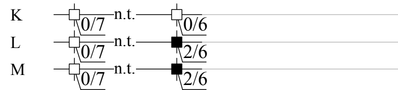


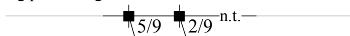
Fig. 2.



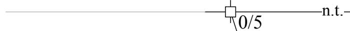
Weaner accommodation farm ff-1



Finishing pen farm gf-1



Slaughterhouse



Supplemental pigs on transportation

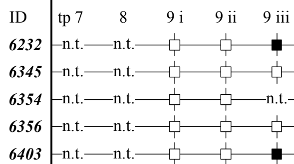


Fig. 3.

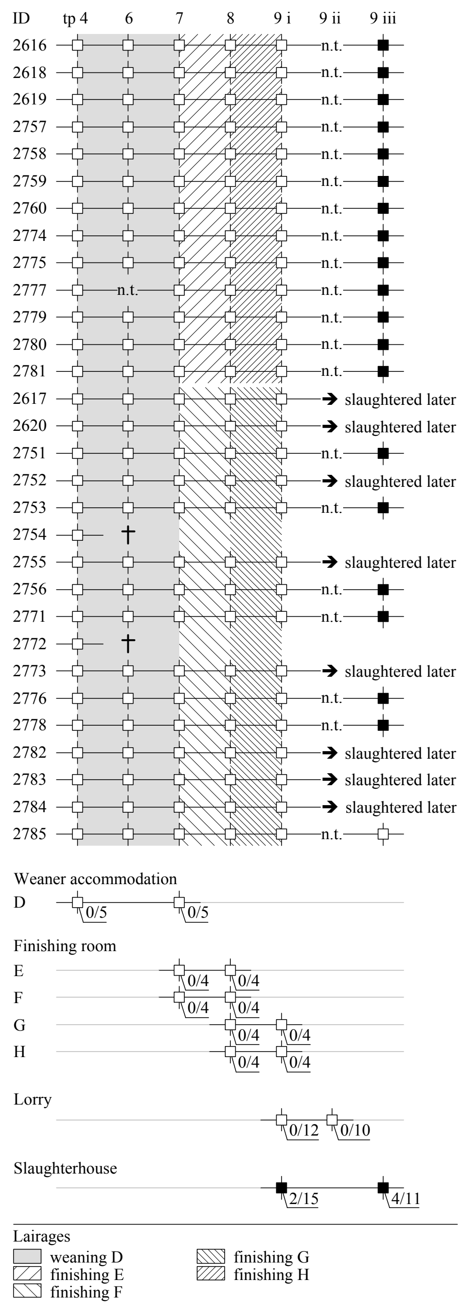


Fig. 4.

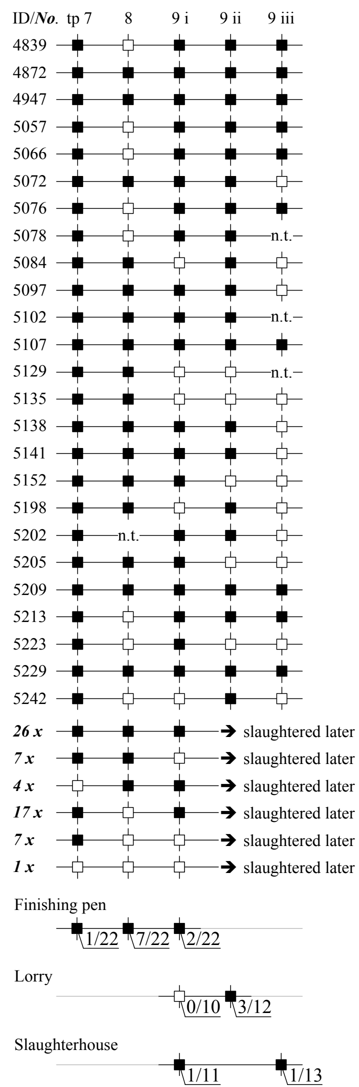


Fig. 5.

	ID/***No.***	tp 7	8	9 i	9 ii	9 iii
farm 1	2126	n.t.	n.t.	□	□	■
	2172	n.t.	n.t.	□	□	■
	2196	n.t.	n.t.	□	■	□
	2245	n.t.	n.t.	■	□	□
	2535	n.t.	n.t.	■	■	■
	2545	n.t.	n.t.	□	n.t.	■
	2557	n.t.	n.t.	□	□	n.t.
	2581	n.t.	n.t.	■	■	□
	2585	n.t.	n.t.	□	■	■
	2600	n.t.	n.t.	□	□	■
	2912	n.t.	n.t.	■	□	■
	2913	n.t.	n.t.	■	■	■
	2914	n.t.	n.t.	□	■	■
	4293	n.t.	n.t.	■	■	■
	4322	n.t.	n.t.	■	□	■
	4325	n.t.	n.t.	□	■	□
	4329	n.t.	n.t.	□	n.t.	■
	4399	n.t.	n.t.	□	■	■
	4406	n.t.	n.t.	■	■	n.t.
	4409	n.t.	n.t.	□	□	■
	7222	n.t.	n.t.	□	■	■
	7250	n.t.	n.t.	□	□	■
	7264	n.t.	n.t.	□	□	■
	7266	n.t.	n.t.	□	■	□
	7269	n.t.	n.t.	□	■	n.t.
	7280	n.t.	n.t.	□	■	□
	7282	n.t.	n.t.	□	□	■
	7284	n.t.	n.t.	□	□	■
	7310	n.t.	n.t.	□	■	■
	7338	n.t.	n.t.	□	□	n.t.
	7343	n.t.	n.t.	□	□	n.t.
	7344	n.t.	n.t.	□	■	■
	7353	n.t.	n.t.	□	■	□
	7358	n.t.	n.t.	□	□	■
	7368	n.t.	n.t.	□	□	■
	n.e.	n.t.	n.t.	□	□	■
	20 x	n.t.	n.t.	□	□	□
farm 2	3092	n.t.	n.t.	□	□	■
	3190	n.t.	n.t.	□	□	n.t.
	3193	n.t.	n.t.	□	■	□
	3196	n.t.	n.t.	□	□	■
	3226	n.t.	n.t.	□	□	■
	3233	n.t.	n.t.	□	□	■
	3254	n.t.	n.t.	□	□	■
	14 x	n.t.	n.t.	□	□	□

Fig. 6.

Acknowledgements

First and foremost I would like to thank Gudrun Overesch for giving me the opportunity to carry out my doctoral thesis at the Institute of Veterinary Bacteriology and for her continuous trust and help. She has answered my many questions constantly with patience and gave me precious advices and particularly for her carefully editing of my manuscript.

I am also grateful to all farmers and staff who granted access to their farms and their kind cooperation; to the slaughterhouse managers and their team, who provide the access to the slaughterhouses and the organisation of taking samples from animals and their facility; to the lorry-drivers for their patience and their permission taking samples of the vehicle; to Patricia Hirsiger, Bettina Jenny, Julia Malik, Dolf Kümmerlen and Wolfgang Pendl, Vetsuisse Faculty, University of Zurich, Switzerland, Saria In-Albon, Julia Furrer, Bettina Meuli, Claudio Dolder and Thomas Mock, Vetsuisse Faculty, University of Berne, Switzerland, for their assistance in taking samples on farms and slaughterhouse; and to Isabelle Brodard and Susanne Rickli, Vetsuisse Faculty, University of Berne, Switzerland, for excellent technical support.

And I thank my family and my grandfather for their support and being empathetic and have helped me realising my dream.

This study was financed by a research grant of Federal Food Safety and Veterinary Office, grant number 1.14.k.

Curriculum Vitae

Name	Patrick Daniel Bangerter
Date of birth	04.07.1974
Place of birth	Bern, Switzerland
Nationality	Swiss
Place of origin	Lyss BE, Switzerland

08/1991 – 07/1998	Vocational training as an architectural drafter and subsequent employments, Burgdorf, Switzerland
08/1998 – 04/1999	Language school, New Zealand
07/1999 – 06/2000	Higher secondary school, Feusi Maturitätsschulen Bern, Switzerland
06.07.2000	Certificate Higher secondary school, Feusi Maturitätsschulen Bern, Switzerland
09/2000 – 02/2004	Undergraduate studies in Architecture, University of Applied Sciences Burgdorf, Switzerland
06.02.2004	Diploma Bachelor of Arts in Architecture, University of Applied Sciences Burgdorf, Switzerland
03/2004 – 08/2008	Employments as an architect, Kirchberg and Bern, Switzerland
09/2008 – 06/2009	Undergraduate studies in Biology, University of Bern, Switzerland
09/2009 – 12/2014	Undergraduate studies Veterinary Medicine, Vetsuisse Faculty, University of Bern, Switzerland
30.12.2014	Diploma med. vet., Vetsuisse Faculty, University of Bern, Switzerland
since 05/2014	Doctoral thesis under the supervision of Dr. Gudrun Overesch at the Institute of Veterinary Bacteriology, Vetsuisse Faculty, University of Bern, Switzerland

Bern, Signature doctoral candidate