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Prevalence and antimicrobial resistance of opportunistic pathogens associated with bovine respiratory disease isolated from nasopharyngeal swabs of veal calves in Switzerland

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ABSTRACT

The composition of the bacterial flora in the calf nasopharynx might influence the risk of bovine respiratory disease (BRD). The aims of the present study were, firstly, to investigate the prevalence of bacteria potentially involved in BRD in the nasopharynx of veal calves and to identify associated risk factors for their presence, and, secondly, to provide data on antimicrobial resistance levels in these bacteria.

Deep nasopharyngeal swabs were collected from veal calves on 12 Swiss farms over a period of one year by non-random, but systematic sampling for isolation of *Pasteurellaceae* and *Mycoplasma* (*M.*) *bovis* and *dispar*. Associations of potential risk factors with occurrence of these bacteria were tested in multivariable mixed logistic regression analyses, based on information gained from extensive questionnaires completed with the farmers. Antimicrobial susceptibility testing was performed for *Pasteurellaceae* by broth microdilution method to obtain minimal inhibitory concentrations (MIC).

Pasteurellaceae, including *Pasteurella* (*P.*) *multocida*, *Mannheimia* (*M.*) *haemolytica*, Bisgaard Taxon 39 and *Histophilus* (*H.*) *somni*, were almost twice as prevalent as *M. bovis* and *dispar* in this study. Continuous stocking was a risk factor for the presence of *Pasteurellaceae*, especially when calves originated from more than six suppliers. In young calves ($\leq$ 91 days), feeding of California Mastitis Test (CMT) positive milk was an additional risk factor for the presence of *Pasteurellaceae* whereas transport of calves by farmers and livestock traders (as opposed to transport only by farmers) increased the risk in older calves ($>$ 91 days). Risk factors for the presence of *M. bovis/dispar* were higher number of calves per drinking nipple in young calves, and no access to an outside pen and feeding of CMT positive milk in older calves, respectively. While further research will have to investigate the observed associations in more detail, this suggests that management can play an important role in the prevalence of nasopharyngeal bacteria with a potential subsequent involvement in BRD. Antimicrobial resistance differed between the three bacterial species tested in this study and was highest to oxytetracycline and spectinomycin in *P. multocida*, oxytetracycline and penicillin in *M. haemolytica,* and ampicillin and penicillin in *H. somni*. Only two European VetCAST breakpoints (for florfenicol in *P. multocida* and *M. haemolytica*) have been published to date, matching the MIC distribution of the present isolate populations well, in contrast to certain commonly applied American Clinical and Laboratory Institute interpretive criteria. This highlights the potential for further refinement of clinical breakpoints in veterinary medicine.

1. Introduction

Bovine respiratory disease (BRD) continues to be one of the main threats to young cattle's lives and has a major financial impact on various cattle production systems (Delabouglise et al., 2017; Wang et al., 2018). Costs result not only from mortality and reduced weaning or carcass weight and quality, but also from expenses for treatment (Pardon et al., 2013; Wang et al., 2018). Calf pneumonia was given as the principal cause of death by 50 % of Swiss veal farmers producing under improved welfare conditions when questioned about mortality in

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their farm (Lava et al., 2016b). It was also the main indication for antimicrobial treatment in veal calves in another study (Schnyder et al., 2019a). Veal calves are particularly at risk of being affected by BRD because of the management factors accompanying traditional veal farming, such as purchase and transport, commingling, and continuous arrival of new animals, which have been associated with increased mortality (Lava et al., 2016a; Schnyder et al., 2019a).

The pathogenesis of the BRD complex has been studied extensively. It is understood as a multifactorial process involving a range of viruses and bacteria as well as environmental and host factors. Stress or viral infections create a basis for opportunistic bacteria to proliferate and populate the lung, and cause bronchopneumonia (Confer, 2009; Guterbock, 2014). Commonly reported viral agents include bovine herpesvirus type 1, bovine parainfluenza-3, bovine viral diarrhoea virus and bovine respiratory syncytial virus. *Mannheimia* (*M.*) *haemolytica*, *Pasteurella* (*P.*) *multocida*, *Histophilus* (*H.*) *somni* and *Mycoplasma* (*M.*) *bovis* are often described as secondary infectious agents but are also commonly considered commensal organisms in the upper bovine respiratory tract. The role of mycoplasmas as potential primary pathogens is subject to discussion despite their common isolation from the respiratory tract of animals without clinical disease (Apley, 2006; Caswell and Archambault, 2007; Griffin et al., 2010; Maunsell et al., 2011; Guterbock, 2014; Grissett et al., 2015). The composition of the bacterial microbiota of the nasopharynx differs between healthy cattle and those affected by BRD, and the presence or absence of certain bacteria in the upper airways might influence the health risk (Holman et al., 2015; Timsit et al., 2018; McMullen et al., 2019). Current data on the prevalence of bacterial commensals with a possible involvement in BRD in the nasopharynx of a large veal calf population are not available. Likewise, management-related factors associated with the prevalence of those bacteria are largely unknown.

Prophylactic measures against BRD include vaccination and antimicrobial therapy upon arrival of the animals in the fattening unit (Pardon et al., 2012; Lava et al., 2016a, 2016b). Both vaccination and antimicrobial mass medication have been associated with decreased mortality (Bähler et al., 2012; Lava et al., 2016b; Baptiste and Kyvsgaard, 2017). However, due to growing concerns about increasing bacterial resistance to antimicrobials, up-to-date information on antimicrobial susceptibility of involved pathogens is needed. Such resistance data provide valuable supporting information for targeted individual treatment rather than prophylactic or metaphylactic group antibiosis (WHO, 2014; EFSA and ECDC, 2019; Schönecker et al., 2019).

The aims of this study were therefore to determine, firstly, the prevalence of *Pasteurellaceae* and *M. bovis/dispar* in the nasopharynx of veal calves of all ages from different farms in Switzerland, and, secondly, to explore possible associations with farm management and related factors. Finally, antimicrobial susceptibility levels of the isolated *P. multocida*, *M. haemolytica* and *H. somni* were investigated to provide information to support the implementation of the principles of a prudent use of antimicrobials in practice.

2. Materials and Methods

2.1. Study design

Twelve farms with an expected minimum of 100 fattened calves per year based on numbers from previous years were recruited for a prospective longitudinal study on veal calf health and antimicrobial resistance in BRD pathogens (Schnyder et al., 2019a; Schönecker et al., 2019). Each farm was monitored for 12 months between July 2016 and November 2017 and visited six times for sampling of veal calves and completion of questionnaires with the farmer. One farm was visited eight times because of shorter fattening periods. Seven farms were run with an all-in/all-out stocking method, while new animals were integrated into the fattening groups continuously throughout the year on the five remaining farms. Calves had an age of 35 days at arrival at the fattening unit on average, and fattening duration ranged from 100 to 127 days (mean 113.8 ± 9.3). The participating farms' capacities differed, as reflected in the maximal number of calves housed on the farms at any time ranging from 35 to 240 (median 53.5; interquartile range, IQR = 45–83.75). Calves were fattened in pens with or without access to an outside area (six farms each) with a maximal group size ranging from 18 to 50 animals. Extensive questionnaires covering the following topics were completed with the farmers during each farm visit: housing of the calves, stocking method and associated factors, hygiene, feeding, and prophylactic measures. Additionally, individual animal information needed to calculate the mean duration of the fattening period, the number of fattened calves per year and each animal's age at sampling were retrieved from the Swiss national animal movement database (Tierverkehrsdatenbank, TVD; Table 1).

2.2. Sample Collection and Processing

All procedures were approved by the competent Committee for Animal Welfare and Protection prior to the start of the project (authorisation number BE 63/16).

Sampling was performed on 50 % of all calves present on the farms on the respective sampling day for the collection of deep nasopharyngeal swabs. Strictly speaking, this was a non-random process; however, samples were collected systematically from animals distributed across all pens in a respective farm as well as from young and older calves alike. On five farms with continuous arrival of calves, half of the swabs were taken from the youngest and oldest animals present, respectively. The other seven farms with an all-in/all-out system were visited at the beginning and at the end of three successive fattening periods. Two nasopharyngeal swabs (COPAN Italia SpA, Brescia, Italy) were taken from the same nostril of every animal selected for swabbing after disinfection of the nostril using gauze swabs (Provet AG/ Henry Schein Animal Health, Lyssach, Switzerland) soaked in 70 % propylalcohol (F25-A Feinsprit 2% MEK, Alcosuisse AG, Bern, Switzerland). One swab was subsequently transferred into liquid Amies transportation medium (Axonlab SwabAX, liquid Amies, Axon Lab AG, Baden, Switzerland), and

Table 1
Overview of potential risk factors for the presence of Pasteurellaceae and Mycoplasma bovis/dipar in the nasopharynx of veal calves as derived from the questionnaire and the Swiss national movement database.

Subject	Parameters (Categories)
Housing of the calves	Surface per calf [m^2] (≤ 2.7 / > 2.7)*; access to outside pen (yes / no); physical contact between calves of different pens (yes / no); shared air space with livestock other than veal calves (yes / no); maximum number of calves per pen (≤ 32 / > 32)*; maximum weight difference within a pen [kg] (≤ 30 / > 30)*; number of fattened calves per year (≤ 146 / > 146)*
Stocking method and associated factors	Stocking method (all-in/all-out / continuous); number of birth farms per 10 calves (≤ 6 / > 6)*; calf transport (farmer / farmer and livestock traders); age at purchase [days] (≤ 35.2 / > 35.2)*; health assessment upon arrival (yes / no) †
Hygiene	Cleaning frequency per year (≤ 5 / > 5)*; disinfection of calf pens (yes / no)
Feeding	Feeding of CMT[1]-positive milk (score ≥ 2; yes / no); number of calves per drinking nipple (≤ 12 / > 12)*; access to roughage (yes / no)
Prophylactic measures	Metaphylactic antimicrobial treatment upon arrival (yes / no); vaccination against BRD pathogens (yes / no) †

*Non-categorical herd level parameters were grouped into binary variables based on the respective mean (normally distributed data)/median (not normally distributed data) of the 12 farms.
† Parameter was excluded from analysis because one of the categories was represented by less than five farms.
[1] CMT = California Mastitis Test, score 2 or higher (Kandeel et al., 2018)

a second one was transferred into liquid medium suitable for sampling for mycoplasmas (DeltaSwab VICUM, deltalab, Barcelona, Spain). All samples were transported to the laboratory within 48 h of collection without cooling and were either cultivated within 72 h of sampling or cryopreserved at −80 °C for later cultivation.

For cultivation of *Pasteurellaceae*, 10 µl of liquid Amies-media were spread on Pasteurella Selective Medium (Thermo Fisher Scientific, Pratteln, Switzerland) and incubated at 37 ± 1 °C for 48 ± 6 h in an atmosphere containing 5% CO_2. Phenotypically relevant strains were cultured on BD Trypticase Soy Agar with 5% Sheep Blood (TSA SB) (Becton Dickinson AG, Basel, Switzerland) for a further 48 ± 6 h under the same conditions.

Ten µl of the mycoplasma transport media were spread both on a Mycoplasma/Ureaplasma Agar (Thermo Fisher Scientific, Pratteln, Switzerland), which was found satisfactory for the isolation of *M. bovis* as previously described by Aebi et al. (2012), and a Mycoplasma Experience agar (Mycoplasma Experience, Surrey, United Kingdom) for isolation of *M. dispar*. The incubation period lasted for a minimum of five days at 37 ± 1 °C in an atmosphere containing 5% CO_2. Plates were checked for bacterial growth under the microscope and colonies showing the typical fried-egg phenotype were re-cultured on the respective agar by agar-transfer.

Species identification was performed by Matrix Assisted Laser Desorption Ionisation Time Of Flight Mass Spectrometry (MALDI TOF MS) using the direct transfer protocol according to the manufacturer's instructions (Biotyper 3.0, Bruker Daltonics GmbH, Bremen, Germany). *Pasteurellaceae* isolates were cryopreserved at −80 °C in tryptone soy bouillon containing 30 % glycerol for subsequent antimicrobial susceptibility testing.

2.3. Antimicrobial Susceptibility Testing

Antimicrobial susceptibility testing was performed by broth microdilution for those bacterial species with available guidelines issued by the Clinical and Laboratory Standards Institute (CLSI, 2013): *P. multocida*, *M. haemolytica* and *H. somni*. Sensititre test plates (BOPO6F for *P. multocida* and *M. haemolytica*, NLD1VMON, NLD2VMON and NLD3VMON for *H. somni*, Thermo Fisher Scientific (TREK Diagnostic Systems)) were used as previously described (Schönecker et al., 2019). In brief, *P. multocida* and *M. haemolytica* were regrown from cryopreservation on TSA SB (18−24 h, 37 ± 1 °C, in an atmosphere containing 5% CO_2) and the obtained colonies were used to achieve an inoculum of approximately 5×10^5 cfu/mL in cation adjusted Mueller-Hinton broth (Thermo Fisher Scientific) supplemented with 5% lysed horse blood (Thermo Fisher Scientific). Inoculated BOPO6F plates were incubated aerobically for 18−24 h at 35 ± 2 °C in a humidified chamber. The reference strain *E. coli* ATTC 25922 was used for quality control and showed minimal inhibitory concentrations (MICs) within the acceptable range. *Histophilus somni* isolates were regrown from cryopreservation on Chocolate Agar (Blood Agar No.2 Base, Becton Dickinson AG) (18−24 h, 37 ± 1 °C, in an atmosphere containing 5% CO_2) and an approximate inoculum of 15×10^5 cfu/mL was achieved in *Haemophilus* Test Medium (HTM) Broth (Remel, Lenexa, KS, USA) supplemented with 500 µL 1ysed horse blood (Thermo Fisher Scientific). The inoculum was higher than recommended in the CLSI guidelines because no visible growth in the positive control was obtained with the recommended concentration after 24 h. We therefore followed the methodology used by DeDonder et al. (2016). Plates were sealed prior to incubation in an atmosphere containing 5% CO_2 for 20−24 h at 35 ± 2 °C in a humidified chamber. *Histophilus somni* ATCC 700025 was used for quality control and showed minimal inhibitory concentrations (MICs) within the acceptable range.

2.4. Data Analyses

Isolates were defined as clinically susceptible (S), intermediate (I) or resistant (R) based on their MICs, applying CLSI clinical breakpoints (CLSI, 2018) and the first two recently published VetCAST clinical breakpoints for florfenicol (issued for *P. multocida* and *M. haemolytica* from the cattle respiratory tract; Veterinary European Committee on Antimicrobial Susceptibility Testing (2019). www.eucast.org). Regarding *P. multocida* and *M. haemolytica*, clinical breakpoints matching the test plate's range were available for ceftiofur, dano- and enrofloxacin, tulathromycin (and tilmicosin for *M. haemolytica*), spectinomycin, penicillin, oxytetracycline and florfenicol. For *H. somni*, breakpoints were available for the following antimicrobial agents: ceftiofur, enrofloxacin, tulathromycin, ampicillin and penicillin, tetracycline and florfenicol.

Each animal's age at sampling was calculated based on their identification number and the information retrieved from the TVD. Bacterial isolation results were transformed into a binary outcome on the individual swab level for statistical analyses. Isolation of at least one of the *Pasteurellaceae* or mycoplasmas under study from a respective swab was classified as "positive"; no isolation of those bacteria was classified as "negative". In regard to the herd level parameters recorded during farm visits, those that were non-categorical were grouped into binary variables based on the respective mean (normally distributed data)/median (not normally distributed data) of the 12 farms. They were combined with the isolation data and each animal's age at sampling in a spreadsheet (Excel 2010, Microsoft, Redmont, WA, USA). This spreadsheet was imported into Stata 16 (StataCorp LLC, College Station, Texas, USA). Descriptive statistics included the calculation of the prevalence of each of the bacterial species under study, prevalence of bacterial combinations isolated from the nasopharyngeal swabs, and MIC50, MIC90, and resistance rates for those antimicrobials with clinical breakpoints available for *P. multocida*, *M. haemolytica* and *H. somni* and matching the used plates' test range.

Associations between farm management and associated factors and the prevalence of *Pasteurellaceae* and *M. bovis/dispar* were analysed using mixed logistic regression analysis (separately for *Pasteurellaceae* and *M. bovis/dispar*). The unit of analysis was the individual swab. To account for the bimodal age distribution of the sampled animals, and because 650 animals had been swabbed twice (once at a young age, and a second time at an older age), analysis was performed separately for the young ($n = 1245$) and the older ($n = 1213$) group of animals. Three-level logistic models were run with Stata's melogit function, accounting for clustering at the level of the farm (12 farms) and fattening group (53 groups). Potential risk factors (Table 1) were screened for associations with the presence of *Pasteurellaceae* or *Mycoplasma bovis/dispar* using univariable mixed regression. Only factors with a p-value < 0.2 in the screening were entered into the multivariable analyses. Since all potential risk factors were binary variables, their correlation was checked using the Phi coefficient test. A strong correlation (Phi coefficient $> |0.7|$) was observed for two parameters: stocking method and shared air space with livestock other than veal calves were perfectly correlated (Phi coefficient = 1), with all farms with continuous stocking also having a shared air space. Consequently, those two factors could not be evaluated separately. In the multivariable analysis, a stepwise backwards selection process was used to build the final model, starting with a full model including all potential risk factors identified in the screening process. Variables were kept in the multivariable model if they were either significantly associated with the presence of bacteria ($p < 0.05$), or if they were relevant confounders changing the effect size of another variable by at least 20 %. Interactions between parameters in the final models were also evaluated. The analysis for *Pasteurellaceae* in older calves resulted in two alternative models in which factors "number of fattened calves per year" or "number of calves per drinking nipple", respectively, remained in addition to factors "stocking method" and "calf transport". "Number of fattened calves per year" and "number of calves per drinking nipple" were moderately correlated (Phi coefficient = 0.5). The former model had an AIC of 1351.5 and a BIC of 1377.0 compared to an AIC of 1352.7 and a BIC of 1378.2 of the latter model (Stata command estat ic). Therefore, the model including "number of fattened calves per

year" was selected as the final model.

3. Results

3.1. Prevalence of opportunistic Pathogens in the upper respiratory Tract

For this study, a total of 2473 deep nasopharyngeal swabs were collected from 1823 animals in 12 farms within the study period. *Pasteurellaceae* were isolated from 58 % of those swabs. The isolates were composed of 1145 *P. multocida*, 337 *M. haemolytica*, 269 Bisgaard Taxon 39, and 26 *H. somni*. *Mycoplasma bovis* ($n = 330$), *M. dispar* ($n = 528$), or both were isolated from 32 % of the swabs. At least one of the *Pasteurellaceae* or mycoplasmas under study was detected in 70 % of the swabs, two or more bacterial species were detected in 29 % of the swabs. Sampling resulted in a bimodal age distribution of the swabbed animals, whose age ranged from 10 to 259 days (median 91). The age of 15 animals could not be determined, leaving 2458 swabs for statistical risk factor analysis. The median age of animals with positive swabs was 106 days (IQR = 56–130), the median age of animals with negative swabs was 54 days (IQR = 38–116).

Pasteurella multocida alone was the most frequently isolated bacterial species in the nasopharynx of both young and older calves (Table 2). Apart from Bisgaard Taxon 39 alone, *M. dispar* and *M. bovis* alone, which were isolated twice as frequently from young calves as from older calves, *M. haemolytica*, *P. multocida*, and bacterial combinations were overall more prevalent in the older group. *Histophilus somni* and *M. bovis* were isolated from animals in eight of the 12 study farms each.

3.2. Risk Factor Analysis for Prevalence Data

In the univariable screening, the following parameters were significantly associated with the presence of *Pasteurellaceae* in the nasopharynx of young (≤ 91 days) calves ($p < 0.05$; percentage of positive swabs in both categories of the respective parameter in brackets): shared air space with livestock other than veal calves (yes: 63.8 %, no: 28.2 %) and stocking method (continuous: 63.8 %, all-in/all-out: 28.2 %), age at purchase (≤ 35.2 days: 59.2 %, > 35.2 days: 27.4 %), and feeding of CMT-positive milk (yes: 57.8 %, no: 25.6 %). Number of birth farms per 10 calves (≤ 6: 58.2 %, > 6: 32.6 %), physical contact between calves of different pens (yes: 49.6 %, no: 37.8 %), disinfection (yes: 43.5 %, no: 48.5 %), and surface per calf (≤ 2.7m^2: 50.1 %, > 2.7m^2: 29.4 %) had a $p < 0.2$ but ≥ 0.05. The presence of *Pasteurellaceae* in the nasopharynx of older calves (> 91 days) was significantly associated with number of calves fattened per year (≤ 146: 83.3 %, > 146: 67.5 %), shared air space with livestock other than veal calves (yes: 76 %, no: 66.9 %) and stocking method (continuous: 76 %, all-in/all-out: 66.9 %), disinfection (yes: 68 %, no: 78.9 %), and number of calves per drinking nipple (≤ 12: 83.5 %, > 12: 67.8 %). Calf transport (farmer: 67.5 %, farmer and livestock traders: 74.8 %), age at purchase (≤ 35.2 days: 75.5 %, > 35.2 days: 66.1 %), physical contact between calves of different pens (yes: 72.2 %, no: 69.8 %), metaphylactic antimicrobial treatment upon arrival (yes: 70.3 %, no: 75.3 %) had a $p < 0.2$ but ≥ 0.05. Factors significantly associated with the presence of *M. bovis/dispar* in the nasopharynx of young calves in the univariable screening were access to roughage (yes: 19.9 %, no: 39.1 %) and number of calves per drinking nipple (≤ 12: 17.2 %, > 12: 40.7 %). Maximum number of calves per pen (≤ 32: 24.9 %, > 32: 39.2 %) and metaphylactic antimicrobial treatment upon arrival (yes: 37.6 %, no: 24.6 %) had a $p < 0.2$ but ≥ 0.05. In older calves, the presence of *M. bovis/dispar* in the nasopharynx was not univariably significantly associated with any of the potential risk factors included in this study. Two factors had a $p < 0.2$ but ≥ 0.05: access to an outside pen (yes: 25.4 %, no: 35.8 %) and feeding of CMT-positive milk (yes: 32.4 %, no: 27 %).

The final multivariable models for *Pasteurellaceae* and *M. bovis/dispar* are presented in Tables 3–6, respectively. In young calves, the nasopharyngeal presence of *Pasteurellaceae* was associated with a continuous stocking method, and the interaction between stocking method and number of birth farms per 10 calves was also significant. For farms with an all-in/all-out protocol, number of birth farms did not influence the presence of *Pasteurellaceae* in young calves. For farms with continuous arrival of animals, the odds for the presence of *Pasteurellaceae* increased with a high number of birth farms. Not feeding CMT-positive milk decreased the odds ratio for the presence of *Pasteurellaceae*. Odds ratio for the presence of *M. bovis/dispar* in the nasopharynx of young calves was decreased for a lower number of calves (≤ 12) per drinking nipple.

Table 2
Prevalence and combinations of isolated bacteria (Pasteurella multocida, Mannheimia haemolytica, Histophilus somni*, Bisgaard Taxon 39, Mycoplasma bovis, Mycoplasma dispar) from 2458 deep nasopharyngeal swabs taken from veal calves on 12 farms in Switzerland during a period of 12 months with respect to their age at sampling (bimodal distribution; young ≤ 91d, older > 91d).

Bacterial species and combinations isolated from deep nasopharyngeal swabs	Prevalence of isolated bacteria			
	Young (n = 1245)		Older (n = 1213)	
	n[1]	[%][2]	n[1]	[%][2,4]
None of the bacteria under study isolated	478	38.4	250	20.6
P. multocida	187	15.0	363	29.9
M. haemolytica	21	1.7	57	4.7
Bisgaard Taxon 39	62	5.0	35	2.9
M. dispar	155	12.5	73	6.0
M. bovis	37	3.0	20	1.6
P. multocida* + *M. haemolytica	34	2.7	85	7.0
***P. multocida* + Bisgaard Taxon 39**	25	2.0	33	2.7
P. multocida* + *M. dispar	44	3.5	85	7.0
P. multocida* + *M. bovis	77	6.2	59	4.9
M. haemolytica* + *M. dispar	N/A[3]		15	1.2
Bisgaard Taxon 39 + *M. dispar*	19	1.5	15	1.2
M. dispar* + *M. bovis	14	1.1	N/A[3]	
P. multocida* + *M. haemolytica* + *M. dispar	N/A[3]		33	2.7
P. multocida* + *M. haemolytica* + *M. bovis	14	1.1	18	1.5
Other combinations[3]	78	6.3	72	5.9

* Not included in the table as only10 *H. somni* were isolated in the young group and 16 *H. somni* in the older group (see supplementary material, Table 1).
N/A = not applicable.
[1] n = number of swabs with the respective bacterial species or combination isolated.
[2] Proportion in regard to the total number of swabs collected from young and older calves, respectively.
[3] Isolated only in low percentages (range: 0.1−0.8%).
[4] Numbers add up to 99.8 instead of 100 because of mathematical rounding.

Table 3
Results from the multivariable mixed logistic regression analysis of the associations between the presence of*Pasteurellaceae* in 1245 deep nasopharyngeal swabs from young veal calves (≤ 91 days) and risk factors identified in 53 fattening groups on 12 veal calf farms in Switzerland.

Parameter	Categories	Odds Ratio	95 % CI		p-value
			lower	**upper**	
Stocking method and number of birth farms per 10 calves	All-in/all-out and > 6 birth farms per 10 calves	Reference			
	All-in/all-out and ≤ 6 birth farms per 10 calves	0.22	0.03	1.74	0.150
	Continuous and > 6 birth farms per 10 calves	8.69	1.43	52.99	0.019
	Continuous and ≤ 6 birth farms per 10 calves	3.61	1.31	9.94	0.013
Feeding of CMT positive milk*	Yes	Reference			
	No	0.35	0.13	0.96	0.041

* CMT score ≥ 2.

Table 4
Results from the multivariable mixed logistic regression analysis of the associations between the presence of *Pasteurellaceae*in 1213 deep nasopharyngeal swabs from older veal calves (> 91 days) and risk factors identified in 53 fattening groups on 12 veal calf farms in Switzerland.

Parameter	Categories	Odds Ratio	95 % CI		*p*-value
			lower	upper	
Stocking method and number of fattened calves per year	All-in/all-out and > 146 calves per year	Reference			
	All-in/all-out and ≤ 146 calves per year	1.82	0.85	3.89	0.124
	Continuous and > 146 calves per year	2.34	1.01	5.40	0.047
	Continuous and ≤ 146 calves per year	7.07	3.10	16.14	<0.001
Calf transport	Farmer	Reference			
	Farmer and livestock traders	2.17	1.06	4.43	0.033

Table 5
Results from the multivariable mixed logistic regression analysis of the associations between the presence of *Mycoplasma bovis/dispar* in 1245 deep nasopharyngeal swabs from young veal calves (≤ 91 days) and risk factors identified in 53 fattening groups on 12 veal calf farms in Switzerland.

Parameter	Categories	Odds Ratio	95 % CI		*p*-value
			lower	upper	
Number of calves per drinking nipple	> 12 calves per nipple	Reference			
	≤ 12 calves per nipple	0.34	0.16	0.76	0.008

Table 6
Results from the multivariable mixed logistic regression analysis of the associations between the presence of *Mycoplasma bovis/dispar* in 1213 deep nasopharyngeal swabs from older veal calves (> 91 days) and risk factors identified in 53 fattening groups on 12 veal calf farms in Switzerland.

Parameter	Categories	Odds Ratio	95 % CI		*p*-value
			lower	upper	
Access to outside pen	Yes	Reference			
	No	1.74	1.10	2.74	0.018
Feeding of CMT positive milk*	Yes	Reference			
	No	0.61	0.38	0.99	0.043

* CMT score ≥ 2.

For older calves, the presence of *Pasteurellaceae* in the nasopharynx was significantly associated with continuous arrival of animals, a lower number of fattened calves per year and calf transport by a transport company. Stocking method interacted with the number of fattened calves in that farms with continuous arrival and a lower number of fattened animals per year were at highest risk for *Pasteurellaceae*. In all-in/all-out farms, the number of fattened animals per year did not have a significant effect. Odds ratios for the presence of *M. bovis/dispar* in older calves was increased for calves not having access to an outside pen and decreased for farms not feeding CMT-positive milk.

3.3. Antimicrobial Susceptibility

Results from the antimicrobial susceptibility testing for *P. multocida*, *M. haemolytica* and *H. somni* are summarised in Tables 7–9, respectively. Based on CLSI breakpoints, the highest observed rates (>80 %) of antimicrobial resistance in *P. multocida* (n = 1145) were against oxytetracycline and spectinomycin, followed by tulathromycin (29 %), penicillin (27 %) and danofloxacin (26 %). Only 5% of the *P. multocida* isolates in this study were fully susceptible to all antimicrobials tested. All *M. haemolytica* (n = 337) were susceptible to ceftiofur, florfenicol and tulathromycin, whereas antimicrobial resistance was most common to oxytetracycline (27 %) and penicillin (26 %). Antimicrobial resistance in *H. somni* (n = 26) was observed to ampicillin (42 %) and penicillin (23 %). None of the *H. somni* isolates was resistant to any of the other evaluated antimicrobials, but 23 % of strains showed an intermediate MIC to tetracycline. Three strains of *H. somni* (12 %) were fully susceptible to all evaluated antimicrobials.

Compared to the interpretation according to CLSI, applying VetCAST breakpoints increased the resistance rate of florfenicol in *P. multocida* from 0.1 to 2%..

4. Discussion

For this study, deep nasopharyngeal swabs were collected from veal calves of various ages on 12 farms. Deep nasopharyngeal swabbing was chosen as diagnostic tool in this study as it is easily applicable to large groups, keeping the stress for the individual animal at a minimal level while still accurately predicting the presence of respiratory pathogens in calves (Godinho et al., 2007). This method has been reported to be inferior to more invasive techniques such as transtracheal aspiration and non-endoscopic bronchoalveolar lavage in regard to predicting the composition of the bacterial microbiota causing pneumonic problems in beef bulls and preweaned calves (Timsit et al., 2013; Van Driessche et al., 2017). However, the same *P. multocida* and *M. haemolytica* ribotyped profiles in both nasopharyngeal swabs and transtracheal samples in 70 % of matched pairs have been described (DeRosa et al., 2000). Furthermore, a significant positive correlation in the detection of bacteria implicated in BRD between nasopharyngeal swabs and lung tissue samples have been reported in one study using PCR (Klima et al., 2014). There was also substantial agreement for bacterial culture of *P. multocida*, *M. haemolytica*, and *H. somni* between pooled bilateral nasopharyngeal swabs and bronchoalveolar lavage fluids in another (Capik et al., 2017). Stress or viral infections can allow for the propagation of bacterial organisms and inhalation into the lungs where they will cause disease (Confer, 2009). Therefore, assessing the prevalence of commensal opportunistic bacteria with pathogenic potential in the upper respiratory tract of veal calves may, indeed, allow for an estimation of the risk of lung infection.

With 70 % of the swabs positive for at least one of the bacterial species under study, we observed an overall prevalence in veal calves of all ages similar to what has recently been described for transtracheal aspiration samples from feedlot cattle with and without BRD (Timsit et al., 2017). As in our investigation using deep nasopharyngeal swabbing, *P. multocida* was the species most frequently isolated in that study, conducted in cattle older than the veal calves included in our study. The higher prevalence of *P. multocida* compared to other *Pasteurellaceae* was attributed to a potential shift towards *P. multocida* rather than *M. haemolytica* as the prime causing agent of BRD (Timsit et al., 2017). A more balanced prevalence of *P. multocida* and *M. haemolytica* was reported in the nasopharynx of diseased veal calves (Pardon et al., 2011). The health status of sampled animals was not assessed in the present study. However, we observed a twofold rise in the prevalence of *P. multocida* from the young to the older group. Despite their much lower total numbers, the increase of *M. haemolytica* from the young to the older group even surpassed that of *P. multocida*. *Histophilus somni* has been described as being frequently isolated from the diseased respiratory tract of North American feedlot cattle (Klima et al., 2014). In the field population under study, we could only isolate it from a minority of samples and only in certain farms, suggesting that *H. somni* might be present in certain environments but not in others. Others before have observed this discrepancy in the isolation of *H. somni* between samples from feedlot

Table 7
MIC distribution of 1145 *P. multocida* isolated from deep nasopharyngeal swabs collected on 12 veal calf farms in Switzerland.

Antimicrobial	MIC (µg/ml)												MIC 50% (µg/ml)	MIC 90% (µg/ml)	Resistance rate (%)*
	0.06	0.12	0.25	0.5	1	2	4	8	16	32	64	128			
Ceftiofur			1114	4	2		3	2	20				≤0.25	≤0.25	2
Danofloxacin		610	122	120	219	74							≤0.12	1	26
Enrofloxacin		653	194	105	190	3							≤0.12	1	0.3
Tulathromycin					23	476	295	12	3	1	6	329	4	>64	29
Spectinomycin									46	172	4	923	>64	>64	81
Penicillin		412	259	162	6		1		305				0.25	>8	27
Oxytetracycline				59	5	8	5	9	1059				>8	>8	93
Florfenicol			28	884	211	3	18	1					0.5	1	0.1

White areas indicate the range of dilution steps tested for each antimicrobial agent; values above this range signify MIC values higher than the highest concentration tested; values at the lowest concentration tested indicate MIC values lower or equal to the lowest concentration tested. Vertical lines indicate the breakpoint for susceptible (S) and resistant (R).
* Interpretation according to CLSI clinical breakpoints issued for the cattle respiratory tract: CLSI Performance Standards for Antimicrobial Disk and Dilution Susceptibility Tests for Bacteria Isolated From Animals, 4th ed. CLSI supplement VET08. Wayne, PA: Clinical and Laboratory Standards Institute; 2018.
Ceftiofur: S ≤ 2; I = 4; R≥8.
Danofloxacin: S ≤ 0.25; I = 0.5; R≥1.
Enrofloxacin: S ≤ 0.25; I = .05–1; R≥2.
Tulathromycin: S ≤ 16; I = 32; R≥64.
Spectinomycin: S ≤ 32; I = 64; R≥128.
Penicillin: S ≤ 0.25; I = 0.5; R≥1.
Oxytetracycline: S ≤ 2; I = 4; R≥8.
Florfenicol: S ≤ 2; I = 4; R≥8.

Table 8
MIC distribution of 337 *M. haemolytica* isolated from deep nasopharyngeal swabs collected on 12 veal calf farms in Switzerland.

Antimicrobial	MIC (µg/ml)												MIC 50% (µg/ml)	MIC 90% (µg/ml)	Resistance rate (%)*
	0.06	0.12	0.25	0.5	1	2	4	8	16	32	64	128			
Ceftiofur			333	4									≤0.25	≤0.25	0
Danofloxacin		256	33	47		1							≤0.12	0.5	0.3
Enrofloxacin		253	24	59		1							≤0.12	0.5	0.3
Tilmicosin							63	99	173	2			16	16	0.6
Tulathromycin						4	217	115	1				4	8	0
Spectinomycin								6	155	175	1		32	32	0
Penicillin		79	79	93	21	1		4	60				0.5	>8	26
Oxytetracycline				246					91				≤0.5	>8	27
Florfenicol			2	91	242	2							1	1	0

White areas indicate the range of dilution steps tested for each antimicrobial agent; values above this range signify MIC values higher than the highest concentration tested; values at the lowest concentration tested indicate MIC values lower or equal to the lowest concentration tested. Vertical lines indicate the breakpoint for susceptible (S) and resistant (R).
* Interpretation according to CLSI clinical breakpoints issued for the cattle respiratory tract: CLSI Performance Standards for Antimicrobial Disk and Dilution Susceptibility Tests for Bacteria Isolated From Animals, 4th ed. CLSI supplement VET08. Wayne, PA: Clinical and Laboratory Standards Institute; 2018.
Ceftiofur: S ≤ 2; I = 4; R≥8.
Danofloxacin: S ≤ 0.25; I = 0.5; R≥1.
Enrofloxacin: S ≤ 0.25; I = 0.5–1; R≥2.
Tilmicosin: S ≤ 8; I = 16; R≥32.
Tulathromycin: S ≤ 16; I = 32; R≥64.
Spectinomycin: S ≤ 32; I = 64; R≥128.
Penicillin: S ≤ 0.25; I = 0.5; R≥1.
Oxytetracycline: S ≤ 2; I = 4; R≥8.
Florfenicol: S ≤ 2; I = 4; R≥8.

cattle in North America and samples from veal calves in Europe (Pardon et al., 2011). When comparing these findings, one should take into account that the North American feedlot production system differs from European veal calf farming and from Swiss veal calf fattening in particular, which is generally performed on a smaller scale compared to the international market (Sans and de Fontguyon, 2009; Lava et al., 2016a).

The prevalence of mycoplasmas in calves with BRD has been said to be likely underestimated since other bacteria involved in the disease grow faster (Nicholas and Ayling, 2003). In the present study, *Pasteurellaceae* and *M. bovis/dispar* were isolated from separate swabs with different transport media; therefore, isolation of one species did not influence isolation of the other. *Mycoplasma bovis* is nowadays regarded by some as a primary pathogen of BRD and the involvement of other *Mycoplasma* species in respiratory disease is being discussed (Nicholas and Ayling, 2003; Angen et al., 2009; Griffin et al., 2010; Nicholas, 2011). Similar to *H. somni*, *M. bovis* was not isolated in all farms in this study. Alone, they were more prevalent in young calves (3.0 % vs. 1.6 % in older calves), which is in accordance with a previous study where young stock was proposed as a potential reservoir for *M. bovis* (Aebi et al., 2015). Similarly, *M. dispar* alone was more prevalent in the nasopharynx of young calves in the present study. While isolated from both healthy and bronchopneumonic feedlot cattle, *M. dispar* has been reported as more abundant in healthy animals (Timsit et al., 2018).

Table 9
MIC distribution of 26 H. somni isolated from deep nasopharyngeal swabs collected on 12 veal calf farms in Switzerland.

Antimicrobial	MIC (µg/ml)																		MIC 50% (µg/ml)	MIC (µg
	0.004	0.008	0.015	0.03	0.06	0.12	0.25	0.5	1	2	4	8	16	32	64	128	256	512		
Ceftiofur				4	2	9	9	2											0.12	0.
Enrofloxacin				24	2														0.03	0.
Tulathromycin									2	18	6								2	
Ampicillin				4	6	5	5					1		4	1				0.12	3
Penicillin			6	4	4	3	3						1	5					0.06	3
Tetracycline									19	1	6								1	
Florfenicol							26												0.25	0.

White areas indicate the range of dilution steps tested for each antimicrobial agent; values above this range signify MIC values higher than the highest concentration tested; values at the lowest concentration tested indicate MIC values lower or equal to the lowest concentration tested. Vertical lines indicate the breakpoint for susceptible (S) and resistant (R).
* Interpretation according to CLSI clinical breakpoints issued for the cattle respiratory tract: CLSI Performance Standards for Antimicrobial Disk and Dilution Susceptibility Tests for Bacteria Isolated From Animals, 4th ed. CLSI supplement VET08. Wayne, PA: Clinical and Laboratory Standards Institute; 2018.
Ceftiofur: S $\leq$ 2; I = 4; R$\geq$8.
Enrofloxacin: S $\leq$ 0.25; I = 0.5–1; R$\geq$2.
Tulathromycin: S $\leq$ 16; I = 32; R$\geq$64.
Ampicillin: S $\leq$ 0.03; I = 0.06−0.12; R $\geq$ 0.25.
Penicillin: S $\leq$ 0.25; I = 0.5; R$\geq$1.
Tetracycline: S $\leq$ 2; I = 4; R$\geq$8.
Florfenicol: S $\leq$ 2; I = 4; R$\geq$8.

Besides those well-defined organisms, another member of the *Pasteurellaceae*, Bisgaard Taxon 39, was isolated from our nasopharyngeal swabs in high numbers. Its role in respiratory disease in calves still has not been defined (Blackall et al., 2001), but similarly to the mycoplasmas under study it was more prevalent in the nasopharynx of young veal calves. Further research regarding its relevance as well as its taxonomic classification is needed.

Risk factor analysis was performed separately for young and older calves in this study using the median age of 91 days as cut-off. As outlined before, sampling had resulted in a bimodal age distribution of the study population. While our cut-off may not be based on immunological data, the two resulting groups may certainly differ in regard to their immunological status; while T-cell populations are present at levels comparable with adult cattle's values, B-lymphocyte numbers increase significantly within the first five months of a calf's life (Kampen et al., 2006). Humoral immunity mediated by B-cells is of particular importance in the protection against bacterial pathogens (Tizard, 2017). Passive maternal immunity confers protection only for the first approximately two to four weeks of a calf's life (McGee and Earley, 2019), leaving veal calves unprotected at an age when they just have been transported and integrated into the fattening group (Bähler et al., 2012). Therefore, assessing risk factors separately for the different age groups makes sense.

In calves up to 91 days of age, continuous arrival of animals at the fattening unit (in interaction with a number of > 6 birth farms per 10 purchased calves) increased the odds ratio (OR) for *Pasteurellaceae* in the nasopharynx, whereas not feeding CMT-positive milk decreased the OR. In older calves, a continuous stocking method was also associated with increased OR for the presence of *Pasteurellaceae*, for this group of animals in interaction with a lower number of fattened calves per year. The interactions with stocking method in the models for young and older calves appear contradictorily at first sight because both parameters (number of birth farms per 10 calves and number of fattened calves per year, respectively) would seem to positively correlate with farm size. However, the two farms with the lowest numbers of fattened calves per year actually had the highest numbers of birth farms per 10 calves, and the farm with the highest number of animals per year had the lowest number of birth farms per 10 calves. Overall, though, number of birth farms per 10 calves and number of fattened calves per year were not correlated (Phi coefficient -0.17).

In older calves the factor "calf transport by farmer and livestock traders" was also associated with an increased OR for the presence of *Pasteurellaceae*. The occurrence of this latter association in older calves rather than the young group was surprising. A possible explanation could be that the new arrivals on a farm introduce a fresh bacterial load into herds, which manifests itself in the older calves, however, further research on the effect of different modes of animal purchase is needed to fully understand this association. Calf purchase in general has been associated with an increased risk for mortality rates above the average in Swiss veal calves before (Lava et al., 2016a) and has also been identified as one of the most important risk factors for pneumonia in young dairy calves (Van Der Fels-Klerx et al., 2000).

A higher number of birth farms has been associated with higher treatment incidences (with BRD as the main indication; Lava et al., 2016b; Schnyder et al., 2019a), and comingling of calves from various sources has been associated with an increased incidence of respiratory disease before (O'Connor et al., 2005; Sanderson et al., 2008). The "how and when" of this mixing are of course important factors to be considered and sudden mixing of animals from various sources without preconditioning has the highest potential negative effect on health (Step et al., 2008; Hay et al., 2014). Such mingling is common practice for veal calf transportation, especially if livestock traders are involved (Schnyder et al., 2019b).

Continuous arrival of calves at the fattening unit was perfectly correlated with a shared air space with livestock other than veal calves in our study farms (Phi coefficient = 1). Aerosol and environmental transmission have been associated with the spread of several pathogens involved in respiratory disease, such as *M. haemolytica*, *H. somni* and *M. bovis* (Jánosi et al., 2009; Klima et al., 2011; Maunsell et al., 2011). Moreover, poor air circulation was considered a risk factor for outbreaks of respiratory disease in both young and dairy calves of up to one year of age (Van Der Fels-Klerx et al., 2000). While we were unable to evaluate shared air space in this study individually, we believe it to be an important consideration and potential risk factor that future studies should evaluate.

A higher number of drinking nipples was associated with decreased OR for *M. bovis/dispar* in young calves. As mentioned before, young cattle is suspected of being a reservoir for *M. bovis* (Aebi et al., 2015), therefore their spread in the veal calf population via highly frequented drinking nipples would be a logical consequence. Odds ratio for *M. bovis/dispar* in older calves was decreased by not feeding CMT-positive milk and increased for calves not having access to an outside pen. Access to an outside pen has been associated with increased mortality and increased treatment incidence in Swiss veal calves before,

which was attributed to likely draft exposure (Lava et al., 2016a; Schnyder et al., 2019a). Taking into account that penicillins (not effective against mycoplasmas) are among the antimicrobials with the highest use in veal calves in Switzerland (Lava et al., 2016b; Schönecker et al., 2019), the reverse association of no outside pen with *M. bovis/dispar* does not come as much as a surprise. Animals with access to an outside pen may be able to escape the group and the inside pen air, which (similarly to less frequented drinking nipples in young calves) may reduce the spread of mycoplasmas in a herd.

The prevalence of mastitis associated with *M. bovis* and *Pasteurellaceae* is low in Switzerland (Aebi et al., 2012, 2015; Rüegsegger et al., 2014), therefore this route of transmission to the respiratory tract of veal calves is questionable. However, the feeding of milk from cows with mastitis might be an indicator for a general poor hygiene and management, and the colonisation of tonsils with *M. bovis* following oral inoculation of calves has been shown (Maunsell et al., 2012).

When comparing the present resistance rates to the literature, caution is warranted since data on antimicrobial resistance in veal calves are sparse and the widely applied interpretive criteria from CLSI are only available for some of the commonly tested antimicrobial agents. Furthermore, the strains tested in this study were isolated from animals of unknown health status and independently of potential previous or concurrent antimicrobial treatments. Taking into account only those antimicrobials interpreted using CLSI clinical breakpoints in other studies, previously reported antimicrobial resistance was common to tetracyclines and tulathromycin in *P. multocida* and tetracyclines, tilmicosin and tulathromycin in *M. haemolytica*, whereas *H. somni* was most commonly resistant to penicillin, tetracyclines and tulathromycin (Catry et al., 2016; Anholt et al., 2017; Timsit et al., 2017). In the present study, we observed high rates of *P. multocida* phenotypically resistant to oxytetracycline and spectinomycin and elevated levels of resistant isolates to tulathromycin, penicillin and danofloxacin in the upper respiratory tract. Elevated resistance rates were also observed for oxytetracycline and penicillin in *M. haemolytica* as well as ampicillin and penicillin in *H. somni*, which also expressed intermediate resistance to tetracycline to a similar degree.

Compared to previous studies in Switzerland, the resistance situation in respiratory *Pasteurellaceae* seems to have changed drastically. We observed an overall increase in resistance in *P. multocida*, with higher rates of resistance to danofloxacin (fluoroquinolone) and tulathromycin (macrolide) becoming particularly apparent in our strains, since distinctly less resistance to these antimicrobials had been reported previously in Switzerland (Vogel et al., 2001; Rérat et al., 2012). Besides potential differences in the antimicrobial treatment status of the calves at the time of sample collection, the spread of certain *P. multocida* strains carrying integrative and conjugative elements (ICE)-associated multidrug resistance should be considered as a possible cause for the high observed resistance rate (Stanford et al., 2020). However, this would require molecular characterisation of the isolated strains, which was not performed in the frame of the present study. We observed an overall decrease in resistance in *M. haemolytica* compared to previous studies (Vogel et al., 2001; Rérat et al., 2012), and an apparent shift from tetracycline to beta-lactam resistance in *H. somni* (Vogel et al., 2001). The number of isolates was low in these previous studies and we were able to investigate antimicrobial resistance in a low number of *H. somni* isolates only, which limits the informative value of a trend analysis in antimicrobial resistance. Therefore, further investigation particularly of resistance patterns in *H. somni* is warranted.

Associations between antimicrobial resistance and antimicrobial drug use in dairy and veal calves are well established (Pereira et al., 2014; Catry et al., 2016; Schönecker et al., 2019). Therefore, the extremely high resistance rate to spectinomycin in *P. multocida* was surprising since there is no labelled medicinal product containing spectinomycin for cattle in Switzerland (https://www.vetpharm.uzh.ch). Some spectinomycin resistance phenotypes are due to genes also conferring streptomycin resistance (Kehrenberg et al., 2005). However, products containing streptomycin are only used to certain extent (approximately 11 % of total antimicrobial drug use) in Swiss veal calf operations (Lava et al., 2016b). Even though several genes responsible for spectinomycin resistance have been described in the meantime, they are not always recovered from strains expressing the resistance phenotype (Stanford et al., 2020) indicating that more research into spectinomycin resistance mechanisms is needed to fully understand this resistance dynamic.

Two clinical breakpoints, for florfenicol in *P. multocida* ($R > 1$ μg/mL) and *M. haemolytica* ($R > 2$ μg/mL) and from the cattle respiratory tract, were recently published by the VetCAST committee. For *P. multocida*, the lower VetCAST clinical breakpoint matched the MIC distribution of the isolate population in this study better than the CLSI resistance breakpoint ($R \geq 8$ μg/mL). The latter does not separate the non-wildtype from the wild-type population, defined by the epidemiological cut-off value (ECOFF; 1 μg/mL), but divides the non-wildtype population. Epidemiological cut-off values allow the detection of antimicrobial resistance as a biological phenomenon, manifesting itself in a non-wildtype subpopulation with acquired resistance mechanisms (EUCAST, 2019). While a clinical breakpoint can never be lower than an ECOFF (Toutain et al., 2017), "microbiological resistance" in non-wildtype isolates may or may not be clinically relevant (EUCAST, 2019), therefore not restricting clinical breakpoints from being set at higher values than the ECOFF. However, exemplified by the differences in florfenicol clinical breakpoints for *P. multocida*, the evidence-based approach to the establishment of clinical breakpoints by the VetCAST committee may constitute an alternative to the established CLSI clinical breakpoints in the future.

Compared to *P. multocida*, the *M. haemolytica* florfenicol breakpoints from VetCAST and CLSI correspond well. Considering the MIC distributions of the three *Pasteurellaceae* species under study overall however, certain American CLSI breakpoints seem not quite to match the populations' distributions in this study.

Although currently no ECOFF for penicillin in *M. haemolytica* is defined, the corresponding CLSI clinical breakpoint ($R \geq 1$ μg/mL) seems to split an assumed wildtype population as seen in our isolates (MIC ranges from ≤ 0.12 μg/mL to 2 μg/mL).

Only 26 *H. somni* isolates were tested in this study, however, even this small population showed a clear bimodal MIC distribution for penicillin and ampicillin, indicating wildtype and non-wildtype subpopulations are present despite the low number of isolates tested. No ECOFFs are currently available for *H. somni*, so wildtype/non-wildtype subpopulations can only be assumed. Based on this, the *H. somni* CLSI clinical breakpoint for penicillin ($R \geq 1$ μg/mL) separates an assumed wildtype population from a smaller second population with distinctly higher MICs (assumed non-wildtype isolates). In contrast, the CLSI clinical breakpoint for ampicillin ($R \geq 0.25$ μg/mL) divides a subpopulation of isolates with low MIC values (ranging from 0.03 to 0.25 μg/mL) that is assumed to represent the wildtype population. One may argue, the methods used in this study for broth microdilution of *H. somni* differed from the CLSI protocol and it is not good practice to apply interpretive criteria to MICs that have been obtained by methodologies diverging from the officially recommended ones (Schwarz et al., 2010). However, this "mixing and matching" is unlikely to represent the causing factor for this study's ampicillin wildtype population to be divided by the CLSI ampicillin clinical breakpoint. If this were the case, we would likely have observed a similar effect for other antimicrobial drugs that we tested for as well. In conclusion, these observations highlight the need for further improvements and developments in the area of interpretive criteria for clinical use.

There are some limiting factors to this study, including farm recruitment on a voluntary basis and the distinctiveness of Swiss veal farming in certain characteristics, which limit a generalisation of the findings to other systems. Additionally, recording each sample's origin to the pen-level would have allowed for even more detailed analyses. Taking antimicrobial treatment records on the individual animal level

into consideration would have made the exploration of antimicrobial susceptibility data even more comprehensive but this was beyond the scope of this study as was an investigation of the presence of viral pathogens, which obviously play an important role in the aetiology of BRD besides bacterial organisms as well.

5. Conclusion

Our findings and their discussion allow the conclusion that preventive measures against BRD should be revisited, starting long before actions aimed at limiting the spread of disease, such as pro(/meta)-phylactic antimicrobial treatments. Most, if not all of the bacterial species investigated in this study are opportunistic pathogens that can be present in the nasopharynx as normal commensals. Management improvements in the areas shown to be associated with the presence of bacterial agents in the upper respiratory tract could potentially help minimize the health risk posed to the calves' lower respiratory tract by these organisms if they are allowed to spread among animals and to multiply in their nasopharynx. However, further research will be necessary to investigate these associations and the preventative potential of corrective measures in more detail.

The antimicrobial resistance profiles of potential BRD bacteria confirm elevated to high levels of resistance to several important antimicrobial substances, which underlines again the need for efficient measures to prevent bacterial pneumonia. The most recent available interpretive criteria in veterinary medicine were used in this study. Differences underline the need for the further refinement of clinical breakpoints based on processes such as those of the VetCAST approach.

Declaration of Competing Interest

The authors declare no conflict of interest.

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