

Article

Pathogen Transmission During the Suckling Period: A Study in Cow-Based Calf Rearing Systems

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Simple Summary

Alternative calf rearing systems, such as rearing calves by cows, represent a more natural practice that is increasingly requested by consumers, but their adoption is constrained by unresolved concerns regarding for example the transmission of mastitis pathogens between calves and cows through suckling. In this study, the prevalence and concordance of mastitis-associated pathogens in the milk of cows (dams and foster cows) and saliva of corresponding calves at the time of weaning were investigated. For this purpose, milk samples from 269 cows (dams and foster cows) and saliva samples from 403 corresponding calves were collected on 15 organic dairy farms in northwest Germany. MALDI-TOF and RAPD-PCR were used for species identification and strain typing. Strain matches compatible with transmission were observed only rarely and were detected for *Mammaliicoccus sciuri* and *Staphylococcus aureus*, exclusively in foster cow–calf pairs. Species-level concordance of *Pasteurella multocida* between cow milk and calf saliva was substantially higher than expected by chance, with an observed-to-expected concordance ratio of 21.1, suggesting a non-random cow–calf co-occurrence. This study provides important evidence to support risk-based management recommendations in cow-based calf-rearing systems.

Abstract

Cow-based calf rearing (dam- or foster cow-based) is gaining popularity as a relatively natural husbandry practice. However, direct udder contact by calves creates a bidirectional transmission route for pathogens. The objective of this cross-sectional study was to investigate the prevalence and concordance of mastitis-associated pathogens in the milk of cows and the saliva of corresponding calves at the time of weaning. On 15 organic dairy farms, milk samples from 269 cows and saliva samples from 403 calves were collected at weaning. Pathogen differentiation was performed via Matrix-assisted laser desorption time-of-flight mass spectrometry (MALDI-TOF), and strain typing was additionally conducted via randomly amplified polymorphic DNA-polymerase chain reaction (RAPD-PCR). Dominant pathogens in milk were Non-*aureus*-Staphylococci (NaS)/*Mammaliicoccus* spp. and *Corynebacterium* spp., and in calf saliva, NaS/*Mammaliicoccus* spp. (primarily *M. sciuri*, *S. xylosus*). There were a total of 12 strain concordances, exclusively in foster cow–calf pairs (Fisher’s exact test, $p = 0.012$). The highest observed/expected ratio was found for *P. multocida* (21.1). For *S. aureus*, strain matches were found in 3 foster cow–calf pairs across 2 farms. Direct strain concordance was generally observed rather rarely. The observed strain concordances suggest transmission between cows and calves, particularly for *S. aureus* and *P. multocida*. NaS and *Mammaliicoccus* spp. concordances are likely attributable to a common environmental source.



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Keywords: calf rearing; cow–calf contact; udder health; foster cow; dam; strain typing

1. Introduction

In recent years, cow-based calf rearing systems, either dam- or foster-based, have gained popularity, driven primarily by consumer demand for more natural husbandry practices [1]. In this form of calf rearing, the animals can engage in natural behaviors such as suckling on the udder. Traditionally, however, early separation of cow and calf has been the norm to protect the calves from pathogens and also to use the cows directly for milk production. For dairy farmers, this creates the challenge of balancing consumer expectations and the prevailing farm conditions—all while aiming to maintain high milk yields. Central to this challenge is the udder health situation of the cows. In contrast to the conventional practice of early separation of cows and calves, cow-based calf rearing systems involve direct and prolonged contact between the calf and the udder, which potentially creates a bidirectional pathway for pathogen transmission.

Mastitis, an inflammation of the mammary gland, is among the most common diseases in dairy cows. According to a recent study from Germany, the most prevalent pathogens causing clinical mastitis are *Streptococcus* (*Sc.*) *uberis*, coliforms, and Staphylococci (Non-*aureus*-Staphylococci (NaS), *Staphylococcus* (*S.*) *aureus*) [2]. In farms where calves are allowed to suckle from cows, there is evidence that suckling calves can transmit pathogens to the udder and thus potentially cause mastitis. Köllmann et al. [3] examined milk samples from foster cows and oral cavity samples from the associated suckling calves and looked for matches between the pathogens found by comparing strains. They found the same isolates in the milk and in the oral cavity for *Pasteurella* (*P.*) *multocida* and *S. aureus*, which suggests that transmission of these pathogens via suckling calves is very likely. *P. multocida* is regularly detected in the upper respiratory tract of calves and is often associated with bovine respiratory disease. As a mastitis pathogen in dairy cows, the pathogen appears to play only a very minor role [4]. A study from Switzerland identified *P. multocida* and *S. aureus* as the most common mastitis pathogens in lactating beef cows [5]. The authors discussed that *P. multocida* from the nasopharyngeal cavity of calves could be the cause of intramammary infections in cows. Since calves do not suckle from cows on most dairy farms, this transmission route could also explain why *P. multocida* mastitis rarely occurs in dairy cows. Furthermore, they discussed how *S. aureus* spreads in beef cow herds, as the typical route of spreading in dairy cow herds is during milking. The calf was discussed as a possible vector, as cross-suckling often occurs in beef cow herds.

Previous research on pathogen transmission in cow-based calf rearing systems has been limited in scope. Studies examining multiple dairy farms and comparing different cow-based rearing systems—particularly dam-based and foster-based approaches—are lacking. It is also unclear whether pathogen transmission patterns observed in single-farm studies apply to diverse farm conditions and management practices. Therefore, the aim of this cross-sectional study was to investigate the presence and concordance of mastitis-associated pathogens in the milk of cows and calf saliva at weaning across multiple dairy farms practicing both dam-based and foster-based calf rearing systems.

2. Materials and Methods

2.1. Study Design

This study is part of the project “KäKNatGeP: Kälberaufzucht an der Kuh—natürlich, gesund und praktikabel” (KäKNatGeP: Calf rearing with cows—natural, healthy and practical), granted by the Federal Office for Agriculture and Food (BLE). In this project,

organic dairy farms across Germany are participating voluntarily, and were eligible for inclusion requiring at least two years of experience with cow-based calf rearing systems. Project farms from northwestern Germany were included in the investigations in this study due to their proximity to the Hanover University of Applied Sciences and Arts laboratory.

Within the framework of the project, quarter milk samples were taken from the cows (dams and foster cows) for microbiological diagnostics and somatic cell count (SCC) at the beginning and end of the suckling period. SCC measurements were performed as part of the overall project protocol but were not included in the present analysis, as they were not relevant to the specific objectives of this study. For the dams, the end of the colostrum period (approx. 3–5 days after calving) was defined as the beginning of the suckling period. For the foster cows, this time was defined as the time when new calves came to the foster cow. Sampling at the end of the suckling period took place around weaning. This time varied between the farms (see Table 1). At the end of the suckling period, dry swabs from the oral cavities were also taken from the calves to be weaned. For the purpose of the present analysis, only quarter milk samples collected at the end of the suckling period were considered, as these were used to assess concordance with the pathogens detected in the oral cavity of the corresponding calves. Cows for which milk samples were available only at the end of the suckling period were also included in the analysis, provided that corresponding oral cavity swab samples from their calves were available.

Table 1. Farm characteristics.

Cow–Calf System	Farms (Location, Number of Cows)	Contact Duration	Suckling System
Foster cows	A (SH ¹ , 60 cows)	90–120 days	Permanent contact
	B (LS ² , 400 cows)		
	C (LS, 140 cows)		
	D (NRW ³ , 40 cows)		
Dams	E (SH, 35 cows)	45 days to 120 days, mostly 90 days	Mostly permanent contact, day contact, or short-time variants
	F (SH, 45 cows)		
	G (LS, 350 cows)		
	H (LS, 70 cows)		
	I (NRW, 15 cows)		
	J (NRW, 35 cows)		
Dams and foster cows	K (SH, 30 cows)	4–6 weeks to 90 days	Mostly permanent contact
	L (SH, 50–60 cows)		
	M (NRW, 70 cows)		
	N (NRW, 70 cows)		
	O (SH, 60 cows)		

¹ SH = Schleswig-Holstein, ² Lower Saxony, ³ North Rhine-Westphalia.

2.2. Farms and Animals

15 organic dairy farms were included in this part of the project. These farms are located in Schleswig-Holstein (n = 6), North Rhine-Westphalia (5), and Lower-Saxony (4). The sampling period was between May 2023 and November 2024.

The herds consisted largely of Holstein-Friesian cows. Other breeds included German Black Pied, Holstein-Friesian, Crossbreed, Angler, Red Pied, Fleckvieh and Jersey. Lactation numbers varied between one to ten. Housing, feeding, and management followed the

standard operating procedures of the respective farms and were not modified for the study. Herd sizes ranged from 15 to 400 milking cows (median: 60 cows).

2.3. Quarter Milk Samples

Quarter milk samples were aseptically collected by a veterinarian or a trained farmer following the guidelines of the German Veterinary Medical Association [6]. Disposable gloves were worn during the entire sampling process and disinfected after each cow. At each sampling, the teat apex was disinfected with 70% ethanol, and the first streams of milk were discarded. 10 mL of milk was collected in sterile test tubes, containing boric acid (Ly20) as a preservative [7]. Afterwards, the milk samples were sent to the laboratory at Hanover University of Applied Sciences and Arts for further analysis. Most samples were collected by the veterinarian and were transported to the laboratory in insulated Styropor boxes on cooling elements (ice packs) to maintain a consistently low temperature as recommended by the GVA [6]. Depending on the farm location, transport to the laboratory took approximately one to several hours. The remaining samples were collected by a trained farmer and either shipped to the laboratory by post or frozen on the farm at -18°C and taken on the next visit by the veterinarian. As all samples were preserved with boric acid (Ly20), sample integrity was maintained during transport, including postal shipment [6].

2.4. Oral Cavity Swabs

Concurrent with the collection of the second milk sample from the cows (around weaning), swab samples were obtained from the oral cavity of the associated calves. Sterile dry swabs were utilized for this purpose (Sterile applicators, Boettger GmbH & Co. KG, Bodenmais, Germany). The calves were either restrained in their usual environment by a helper or fixed to the edge of the barn with the help of mobile fence elements. The calves' mouths were then opened, and the sample was taken from the rear area around the tonsils, if possible, by the veterinarian. If the samples were contaminated by too much feed residue, a second sample was taken. The samples were then placed directly into sterile test tubes containing 2 mL of brain–heart broth (Merck KGaA, Darmstadt, Germany). Between the different calves, the mouth wedge was cleaned with iodine soap containing povidone iodine (Novasan Plus Jodseife, Ferd. Eimermacher GmbH and Co. KG, Nordwalde, Germany). The swabs were either transported to the laboratory in cooling boxes for further analysis on the day of sampling or frozen.

2.5. Laboratory Procedures

2.5.1. Quarter Milk Samples

The cytomicrobiological analyses of the milk samples were carried out at the Hannover University of Applied Sciences and Arts. The analysis was based on the guidelines of the GVA [6]. Firstly, 10 μL of each milk sample was plated onto an esculin blood agar plate (5% defibrinated sheep blood, Oxoid Deutschland GmbH, Wesel, Germany). The plates were then aerobically incubated at 37°C and analyzed after 24 h and 48 h. The initial classification relied on the evaluation of colony morphology and hemolysis patterns. Subsequent classification was performed using Gram staining and biochemical assays, such as catalase activity testing (3% H_2O_2 ; Merck KGaA, Darmstadt, Germany) and aesculin hydrolysis.

The bacteriological results were afterwards confirmed using the Matrix-assisted laser desorption time-of-flight mass spectrometry (MALDI-TOF, Bruker Daltonics GmbH & Co. KG, Bremen, Germany). This was performed with a direct transfer method without acid extraction using the MBT Compass library (Revision F, MBT 84,668 MSP Library, Bruker Daltonics GmbH & Co. KG, Bremen, Germany). For pathogens with a MALDI-TOF log score above 2, the pathogen was considered identified to species level. If the log score was between 1.7 and 2, and the species level was not identified by morphological and

biochemical classification, the pathogen was considered identified at genus level. The SomaScope™ Smart (Delta Instruments B.V., Drachten, The Netherlands) was used for the determination of somatic cell count (SCC). The presence of more than two different colony types on a blood agar plate led to the classification of the milk sample as contaminated. If two different pathogens were detected in one sample, the sample was classified as mixed infection.

2.5.2. Oral Cavity Swabs

The examinations of the oral cavity swabs were also carried out at the Hannover University of Applied Sciences and Arts. As a first step, the swabs were vortexed for 30 s (Vortex Genie2, Scientific Industries Inc., Bohemia, NY, USA). Dilution series of 10^{-1} and 10^{-2} were then prepared using Ringer's solution. For samples with visible organic material or expected high bacterial loads, a further dilution step of 10^{-3} was used. The entire surface of an esculin blood agar plate was covered with 100 µL of each sample using a Drigalski spatula. Subsequent incubation took place aerobically at 37 °C for 48 h.

Most of the plates showed growth of many different pathogens after 48 h of incubation. Therefore, in the subsequent bacteriological examination, the focus was placed on pathogens that were also found in the quarter milk samples of the cows (dams and foster cows) and on typical mastitis pathogens; a maximum of 8 isolates were collected in total. As with the milk samples, the bacteriological results of the oral swabs were confirmed using the Matrix-assisted laser desorption time-of-flight mass-spectrometry (MALDI-TOF, Bruker Daltonics GmbH & Co. KG, Bremen, Germany) with a direct transfer method without acid extraction, using the MBT Compass Library (Revision F, MBT 84,668 MSP Library, Bruker Daltonics GmbH & Co. KG, Bremen, Germany). The same MALDI-TOF log score thresholds as described above were applied (log score > 2 for species-level, and 1.7–2 for genus-level identification). Subsequently, all isolates were stored at −80 °C with 800 µL of brain–heart broth (Merck KGaA, Darmstadt, Germany) and 200 µL of glycerol until further use.

2.5.3. Strain Comparison

Strain comparison was performed on all matching pathogens found in the cow–calf combination at the end of the sampling period. This was done with the help of randomly amplified polymorphic DNA-polymerase chain reaction (RAPD-PCR). Bacterial DNA was extracted using the DNeasy Blood and Tissue Kit (Qiagen Benelux B.V., Venlo, The Netherlands) according to the manufacturer's instructions.

The RAPD-PCR was carried out in Mx3005 P qPCR system (Agilent Technologies, Inc., Santa Clara, CA, USA) with a reaction mix containing 12.5 µL of ROTI[®]Pol TaqS Mix (2×) (Carl Roth, Karlsruhe, Germany), 5 µL DNA template, 10 pmol of each primer and distilled deionized water to reach the final volume of 25 µL. The following primers were used: OPE 04 (5'-GTGACATGCC-3') [8], C-Primer (5'-CGGGGGACTGTTGGGCGCCATCT3') [9]. To visualize the PCR products, staining with 1 µL of Midori Green Direct (Nippon Genetics Europe GmbH, Dürren, Germany) and separating by electrophoresis on a 2% agarose gel for 1.5 h at 150 V were performed. A 100 bp DNA ladder (Carl Roth GmbH + Co.KG, Karlsruhe, Germany) was run in each gel as the molecular size marker. Gel pictures were taken with the InGenius LHR-system (Syngene International Ltd., Cambridge, UK). The RAPD patterns that appeared were evaluated using the software GeneSnap (Syngene International Ltd., Cambridge, UK, product version 7.12). RAPD-PCR products that showed identical patterns in the first gel run were grouped together and loaded side by side onto a second gel. If the banding patterns were identical, the isolates were regarded as belonging to the same strain.

This step was performed at the end of the sampling period, as all microorganisms were then present and could be analyzed and compared collectively within each facility.

All isolates from one species were processed simultaneously under identical PCR and electrophoresis conditions to ensure maximum comparability and to minimize potential inter-run variability.

2.6. Statistical Analyses

Data were collected and analyzed using Excel, Office 2010 (Microsoft Corporation, Redmond, WA, USA) and SPSS (IBM SPSS 29.0.0.0, Armonk, NY, USA). The statistical unit was the udder quarter of a cow; in the calculations of concordances, the cow was the statistical unit.

The prevalence of pathogens was calculated at the level of individual milk sample events from cows ($n = 269$), which corresponds to the total number of quarter milk sample sets collected. Cows from which samples were collected at multiple time points were counted as separate sample events. A sample from a cow was considered positive for a specific pathogen if it was detected in at least one udder quarter. The prevalence at the calf level was calculated as the proportion of calves ($n = 403$) that was tested positive for a specific pathogen in the oral cavity sample.

To assess whether the frequency of species-level concordance between cow milk and calf saliva exceeded what would be expected by chance, an expected concordance frequency was calculated for each pathogen as the product of cow-level prevalence, calf-level prevalence, and the total number of cow–calf pairs ($n = 572$). The ratio of observed to expected concordances was used as an indicator of non-random co-occurrence.

The association between concordance (species-level and strain-level) and the variables suckling duration category (1: ≤ 30 days, 2: $>30\text{--}\leq 60$ days, 3: $>60\text{--}\leq 90$ days, 4: >90 days) and cow type (dam vs. foster cow) was assessed using cross-tabulation and Pearson chi-square tests. A significance level of $\alpha = 0.05$ was assumed.

3. Results

Milk samples originated from 237 individual cows. Some of these cows ($n = 26$) were sampled and included multiple times, resulting in a total of 269 cows. These were predominantly foster cows that participated in multiple calf-rearing cycles or dams that were included in two consecutive years. Specifically, one cow was sampled five times (foster cow with five calf-rearing cycles), three cows were sampled three times each, and 22 cows were sampled twice.

The results of 1056 quarter milk samples of the 269 cows (163 dams and 106 foster cows) at the time of weaning are shown in Table 2. A result was missing for 20 quarters. Reasons for this were empty quarters from foster cows from which no milk could be milked because they had been emptied by calves before sampling, as well as non-functioning quarters.

Table 2. Pathogens isolated from quarter milk samples of the cows (dams/foster cows, $n = 269$) around weaning ($n = 1056$ udder quarters).

Pathogen/Pathogen Group	Number (%)	Pathogen Species (n)	
NaS/ <i>Mammaliicoccus</i> spp. ¹	139 (13.16)	<i>Staphylococcus</i> (<i>S.</i>) <i>chromogenes</i>	28 (20.1)
		<i>S. xylosum</i>	25 (18.0)
		<i>S. haemolyticus</i>	21 (15.1)
		<i>Mammaliicoccus sciuri</i>	19 (13.7)
		<i>S. equorum</i>	16 (11.5)
		Other	19 (13.7)
		NaS without species identification	11 (7.9)

Table 2. Cont.

Pathogen/Pathogen Group	Number (%)	Pathogen Species (n)	
<i>Corynebacterium</i> (C.) spp.	114 (10.8)	<i>C. bovis</i>	99 (86.8)
		<i>C. glutamicum</i>	6 (5.3)
		Other	9 (7.9)
<i>Staphylococcus</i> (S.) <i>aureus</i>	34 (3.22)		
<i>Streptococcus</i> spp.	26 (2.46)	<i>Sc. uberis</i>	9 (34.6)
		<i>Sc. dysgalactiae</i>	7 (26.9)
		Other	10 (38.5)
<i>Bacillus</i> spp.	17 (1.61)		
<i>Acinetobacter</i> spp.	8 (0.76)		
<i>Pasteurella</i> (P.) <i>multocida</i>	5 (0.47)		
<i>Lactococcus</i> spp.	3 (0.28)		
<i>Enterococcus</i> spp.	3 (0.28)		
Mixed infections	25 (2.37)		
Others	17 (1.61)		
Contaminated	15 (1.42)		
Pathogen detection in total	406 (38.4)		
No specific growth	650 (61.6)		
Grand total	1056 (100)		

¹ Non-*aureus*-Staphylococci/*Mammaliicoccus* spp. (until recently, *Mammaliicoccus* spp. were formerly included within the NaS group).

Swab samples were collected from a total of 407 calves. Of these, 403 samples were included in the analysis, as four calves were sampled twice. In these cases, calves were still with their dam or foster cow during a subsequent visit, and an additional sample was taken in order to collect material as close as possible to the weaning date; only the latter sample was included in the analysis. The results of the swab samples of the oral cavity of the calves are shown in Table 3.

Table 3. Pathogens isolated from oral cavity samples of the calves around weaning (n = 403).

Pathogen/Pathogen Group	Number (%)	Pathogen Species (n)	
Non- <i>aureus</i> -Staphylococci (NaS) / <i>Mammaliicoccus</i> spp.	894 (26.8)	<i>M. sciuri</i>	261 (29.2)
		<i>S. xylosus</i>	224 (25.1)
		<i>S. equorum</i>	102 (11.4)
		<i>S. haemolyticus</i>	84 (9.4)
		<i>S. succinus</i>	83 (9.3)
		<i>S. vitulinus</i>	61 (6.8)
		<i>S. chromogenes</i>	38 (4.3)
		<i>S. gallinarum</i>	18 (2.0)
		<i>S. auricularis</i>	9 (1.0)
		<i>S. pasteurii</i>	3 (0.3)
		<i>S. cohnii</i>	2 (0.2)

Table 3. Cont.

Pathogen/Pathogen Group	Number (%)	Pathogen Species (n)	
Non-aureus-Staphylococci (NaS) /Mammaliicoccus spp.	894 (26.8)	<i>S. warneri</i>	2 (0.2)
		<i>S. fleurettii</i>	2 (0.2)
		<i>S. hyicus</i>	2 (0.2)
		<i>S. devriesei</i>	1 (0.1)
		<i>S. lentus</i>	1 (0.1)
		NaS without species identification	1 (0.1)
<i>Bacillus</i> spp.	179 (5.4)	<i>B. licheniformis</i>	88 (49.2)
		<i>B. pumilus</i>	56 (31.3)
		<i>B. oleronius</i>	9 (5.0)
		<i>B. cereus</i>	4 (2.2)
		<i>B. clausii</i>	4 (2.2)
		<i>B. altitudinis</i>	3 (1.7)
		<i>B. circulans</i>	3 (1.7)
		<i>B. subtilis</i>	3 (1.7)
		<i>B. kochii</i>	1 (0.6)
		<i>B. simplex</i>	1 (0.6)
		<i>B. oceanisediminis</i>	1 (0.6)
		<i>B. without species identification</i>	6 (3.4)
<i>Streptococcus</i> spp.	174 (5.2)	<i>Sc. pluranimalium</i>	66 (37.9)
		<i>Sc. lutetiensis</i>	23 (13.2)
		<i>Sc. gallolyticus</i>	21 (12.1)
		<i>Sc. suis</i>	18 (10.3)
		<i>Sc. equinus</i>	10 (5.7)
		<i>Sc. pherorimalium</i>	10 (5.7)
		<i>Sc. henryi</i>	9 (5.2)
		<i>Sc. parauberis</i>	4 (2.3)
		<i>Sc. uberis</i>	3 (1.7)
		<i>Sc. dysgalactiae</i>	3 (1.7)
		<i>Sc. mitis</i>	2 (1.1)
		<i>Sc. thoraltensi</i>	2 (1.1)
		<i>Sc. alactolyticus</i>	1 (0.6)
		<i>Sc. minor</i>	1 (0.6)
		<i>Sc. oralis</i>	1 (0.6)
<i>Corynebacterium</i> spp.	136 (4.1)	<i>C. xerosis</i>	53 (29.0)
		<i>C. glutamicum</i>	21 (15.4)
		<i>C. casei</i>	18 (13.2)
		<i>C. camporealensis</i>	11 (8.1)
		<i>C. flavesceus</i>	7 (5.1)
		<i>C. stationis</i>	6 (4.4)

Table 3. Cont.

Pathogen/Pathogen Group	Number (%)	Pathogen Species (n)	
<i>Corynebacterium</i> spp.	136 (4.1)	<i>C. urealyticum</i>	6 (4.4)
		<i>C. callunae</i>	4 (2.9)
		<i>C. efficiens</i>	1 (0.7)
		<i>C. glutamicum</i>	1 (0.7)
		<i>C. stationis</i>	1 (0.7)
		<i>C. without species identification</i>	7 (5.1)
<i>Aerococcus viridans</i>	133 (4.0)		
<i>Acinetobacter</i> spp.	81 (2.4)	<i>A. lwoffii</i>	38 (46.9)
		<i>A. johnsonii</i>	9 (11.1)
		<i>A. baumannii</i>	7 (8.6)
		<i>A. beijerinckii</i>	4 (4.9)
		<i>A. bohemicus</i>	4 (4.9)
		<i>A. proteolyticus</i>	4 (4.9)
		<i>A. puyangsis</i>	3 (3.7)
		<i>A. calcoaceticus</i>	2 (2.5)
		<i>A. indicus</i>	2 (2.5)
		<i>A. guillouiae</i>	1 (1.2)
		<i>Acinetobacter</i> spp. without species identification	7 (8.6)
<i>Rothia</i> spp.	62 (1.9)	<i>R. nasimurium</i>	59 (95.2)
		<i>R. endophytica</i>	3 (4.8)
<i>Pantoea agglomerans</i>	49 (1.5)		
<i>Glutamicibacter</i> spp.	45 (1.3)	<i>G. arilaitensi</i>	35 (77.8)
		<i>G. protophormiae</i>	9 (20.0)
		<i>G. mysorens</i>	1 (2.2)
<i>Enterococcus</i> spp.	41 (1.2)	<i>E. faecalis</i>	8 (19.5)
		<i>E. durans</i>	7 (17.1)
		<i>E. faecium</i>	6 (14.6)
		<i>E. saccharolyticus</i>	6 (14.6)
		<i>E. aquimarinus</i>	5 (12.2)
		<i>E. italicus</i>	3 (7.3)
		<i>E. casseliflavus</i>	2 (4.9)
		<i>E. gallinarum</i>	2 (4.9)
		<i>E. moraviensis</i>	1 (2.4)
		<i>E. mundtii</i>	1 (2.4)
		<i>W. cibaria</i>	24 (61.5)
<i>Weissella</i> spp.	39 (1.2)	<i>W. paramesenteroides</i>	12 (30.8)
		<i>W. hellenica</i>	2 (5.1)
		<i>W. falserui</i>	1 (2.6)

Table 3. Cont.

Pathogen/Pathogen Group	Number (%)	Pathogen Species (n)	
<i>Pseudomonas</i> spp.	36 (1.1)	<i>P. aeruginosa</i>	11 (30.6)
		<i>P. putida</i>	9 (25.0)
		<i>P. koreensis</i>	2 (5.6)
		<i>P. flavescens</i>	2 (5.6)
		<i>P. fluorescens</i>	2 (5.6)
		Other	10 (27.8)
<i>Lactobacillus</i> spp.	36 (1.1)	<i>L. plantarum</i>	27 (75.9)
		<i>L. rhamnosus</i>	6 (16.7)
		<i>L. curvatus</i>	2 (5.6)
		<i>L. raffinolactis</i>	1 (2.8)
<i>Kocuria</i> spp.	33 (1.0)	<i>K. salsicia</i>	13 (39.4)
		<i>K. carniphila</i>	12 (36.4)
		<i>K. palustris</i>	3 (9.1)
		<i>K. rhizophila</i>	2 (6.1)
		<i>K. vatians</i>	2 (6.1)
		<i>K. rosea</i>	1 (3.0)
<i>Globicatella</i> spp.	30 (0.9)	<i>G. sanguinis</i>	24 (80.0)
		<i>G. sulfidifaciens</i>	6 (20.0)
<i>Staphylococcus aureus</i>	27 (0.8)		
<i>Pasteurella multocida</i>	21 (0.6)		
Other	386 (11.6)	<i>Arthrobacter</i> spp.	21 (5.4)
		<i>Microbacterium</i> spp.	21 (5.4)
		<i>Micrococcus</i> spp.	19 (4.9)
		<i>Brachybacterium</i> spp.	14 (3.6)
		<i>Psychrobacter</i> spp.	14 (3.6)
		<i>Serratia</i> spp.	12 (3.1)
		<i>Macrococcus</i> spp.	11 (2.8)
		<i>Enterobacter</i> spp.	9 (2.3)
		<i>Brevundimonas</i> spp.	8 (2.1)
		<i>Mannheimia</i> spp.	8 (2.1)
		Other	238 (61.7)
Identification not possible	935 (28.0)		
Total	3337 (100)		

The mean number of calves per foster cow was 3.69 and per dam 1.11. Overall, 572 cow–calf pairs (391 foster cow–calf pairs vs. 181 dam–calf pairs) with complete milk and saliva samples were available for concordance analysis.

In the first step, it was determined which pathogens showed species matching according to MALDI-TOF between the pathogens found in the oral cavity of a calf and the pathogens found in the milk sample from the corresponding cow. For 116 pathogens, a species match could be found, with *Mammaliicoccus sciuri* being the most frequently

matched pathogen, followed by *S. xylosus*, *Aerococcus viridans*, and *Bacillus* spp. (Table 4). There was one match in each of 98 cow–calf pairs, including 74 foster cow–calf pairs and 24 dam–calf pairs. There were two matches in 7 foster cow–calf pairs and 2 dam–calf pairs.

Table 4. Pathogens for which a species match (MALDI-TOF) and a strain-typing match (RAPD-PCR) was found between the pathogens from the oral cavity of a calf and those from the milk sample of the corresponding cow at the time of weaning.

Pathogen	Species Match (MALDI-TOF ¹)	Strain Comparison (RAPD-PCR ²)		
		Match	Not Reculturable	No Match (Different Strains)
<i>Mammaliicoccus sciuri</i>	35	9	8	18
<i>Staphylococcus (S.) xylosus</i>	17	0	6	11
<i>Aerococcus viridans</i>	12	0	3	9
<i>Bacillus (B.) licheniformis</i>	9	-	-	-
<i>B. pumilus</i>	9	-	-	-
<i>S. chromogenes</i>	7	0	1	6
<i>S. aureus</i>	5	3	1	1
<i>Pasteurella multocida</i>	4	0	4	0
<i>S. equorum</i>	4	0	0	4
<i>S. haemolyticus</i>	4	0	0	4
<i>Streptococcus pluranimalium</i>	4	0	0	4
<i>Corynebacterium xerosis</i>	2	0	0	2
<i>Macrococcus canis</i>	1	-	-	-
<i>Rothia nasimurium</i>	1	-	-	-
<i>S. succinus</i>	1	0	1	0
<i>S. vitulinus</i>	1	0	1	0
Total	116	12	25	59

Blue = Species match; Green = Strain typing match; Red = Isolates lost during storage (could not be recultured for strain typing); Orange = Different strains (no match in RAPD-PCR); Gray (-) = No strain comparison performed.
¹ Matrix-assisted laser desorption time-of-flight mass spectrometry; ² randomly amplified polymorphic DNA-polymerase chain reaction.

In a second step, strain comparison was conducted for the pathogens where a species match was found. Strain comparisons were only performed for microorganisms with proven pathogenicity in the mammary gland. Therefore, this step was not performed for *Bacillus* spp. ($n = 18$), with 9 *Bacillus licheniformis* and 9 *Bacillus pumilus*, as well as one isolate each of *Macrococcus canis* and *Rothia nasimurium*.

The same strain in the milk sample of the cow and the oral cavity sample of the calf was found in 12 cases (Table 4). Nine of 35 *M. sciuri* and three of five *S. aureus* showed identical RAPD PCR patterns. All matches at strain level were found between foster cows and their corresponding calves. The 12 strain-level concordances involved five foster cows across three farms. On farm A, one foster cow shared an identical *S. aureus* strain with two of her calves. On farm B, three foster cows housed in a shared foster cow group each showed strain-level concordance with the same three calves for *M. sciuri*, with all matches detected on a single sampling date. On farm C, one foster cow shared an identical *S. aureus* strain with one calf.

For 25 isolates, no strain comparison could be made because the isolates no longer grew after defrosting. These included both calf and cow isolates. Specifically, eight of *M. sciuri*, six of *S. xylosus*, all four isolates of *P. multocida*, three of *A. viridans*, one each of *S.*

aureus, *S. chromogenes*, *S. succinus*, and *S. vitulinus* were affected. No matches were found for 59 isolates. This included 18 of *M. sciuri*, 11 of *S. xylosus*, nine isolates of *A. viridans*, six of *S. chromogenes*, four of *S. equorum*, four of *S. haemolyticus*, four of *Sc. pluranimalium*, two of *C. xerosis* and one of *S. aureus*.

In some cases, identical strains were found in constellations other than between the cow and the corresponding calf. These included matches of multiple pathogens in one sample from the same animal (cows and calves, for *S. aureus*, *M. sciuri*, *S. xylosus*), between calves sharing the same foster cow (*M. sciuri*, *C. xerosis*), and between different foster cows all being part of one foster cow–calf group (*M. sciuri*).

No significant differences in distribution were observed with respect to species-level concordance and suckling duration category ($\chi^2 = 5.715$, $df = 3$, $p = 0.126$) or cow type (dam vs. foster cow; $\chi^2 = 3.743$, $df = 1$, $p = 0.053$) based on the results of the Chi-square analysis. In contrast, strain-level concordance was significantly associated with cow type (Fisher's exact test, $p = 0.012$). All 12 strain matches were found exclusively in foster cow–calf pairs, with none detected in dam–calf pairs. A significant association was also found between strain-level concordance and the suckling duration category ($\chi^2 = 11.635$, $df = 3$, $p = 0.009$), though this result should be interpreted with caution as 3 of 8 cells had expected frequencies below 5 (minimum expected count: 0.77).

To assess whether a transmission of certain pathogens via cow–calf-interaction reflects their prevalence in cow milk and calf saliva or if there are certain pathogens that are transmitted more often, the prevalence of the pathogens was calculated at the animal level separately for cows ($n = 269$) and calves ($n = 403$) (Table 5). Among the pathogens for which species concordance was observed, meaning they were detected in both milk and saliva, *S. chromogenes* (11.9%), *S. xylosus* (9.7%) and *S. aureus* (9.3%) had the highest prevalence at the cow level. At the calf level, *S. xylosus* (33.3%) and *M. sciuri* (30.5%) were the most prevalent pathogens.

Table 5. Animal-level prevalence of selected pathogens for which a species-level concordance was found in milk of the cows (dams and foster cows, $n = 269$) and the saliva of the calves ($n = 403$).

Pathogen	Prevalence in Milk of the Cows (% , n)	Prevalence in Saliva of Calves (% , n)	Ratio Calf Saliva/Cow Milk
<i>Staphylococcus (S.) vitulinus</i>	0.7 (2)	10.2 (41)	13.7
<i>Streptococcus pluranimalium</i>	0.7 (2)	9.7 (39)	13.0
<i>S. succinus</i>	1.1 (3)	14.4 (58)	12.9
<i>Corynebacterium xerosis</i>	0.7 (2)	6.0 (24)	8.0
<i>Aerococcus viridans</i>	3.7 (10)	18.4 (74)	4.9
<i>Mammaliicoccus sciuri</i>	8.9 (24)	30.5 (123)	3.4
<i>S. xylosus</i>	9.7 (26)	33.3 (134)	3.4
<i>S. equorum</i>	5.2 (14)	13.6 (55)	2.6
<i>Pasteurella multocida</i>	1.5 (4)	2.2 (9)	1.5
<i>S. haemolyticus</i>	8.2 (22)	10.9 (44)	1.3
<i>S. chromogenes</i>	11.9 (32)	6.5 (26)	0.5
<i>S. aureus</i>	9.3 (25)	3.2 (13)	0.3

As a next step, it was determined whether a pathogen is common in the calves' saliva because it is also common in the cows' milk, or whether it is common there independently of that. To this end, expected concordance frequencies for each pathogen were calculated

as the number of matches that would be expected purely by chance if a cow and a calf had the pathogen completely independently of each other. This was calculated as the product of the prevalence at the cow level, the prevalence at the calf level, and the total number of cow–calf pairs ($n = 572$). Subsequently, the ratio of observed to expected matches was calculated. A ratio greater than 1.0 indicates that a pathogen was found in cow–calf pairs more often than expected by chance, suggesting a possible transmission-related association (Table 6). The highest ratio of observed to expected matches were observed for *P. multocida* (21.1), followed by *Sc. pluranimalium* (9.7) and *C. xerosis* (7.9).

Table 6. Observed and expected species-level concordances between cow milk and calf saliva samples ($n = 572$ pairs).

Pathogen	Expected (n)	Observed (n)	Ratio Obs./Exp.
<i>Pasteurella multocida</i>	0.19	4	21.1
<i>Streptococcus pluranimalium</i>	0.41	4	9.7
<i>Corynebacterium xerosis</i>	0.25	2	7.9
<i>Aerococcus viridans</i>	3.9	12	3.1
<i>Staphylococcus</i> (S.) <i>aureus</i>	1.71	5	2.9
<i>S. vitulinus</i>	0.43	1	2.3
<i>Mammaliicoccus sciuri</i>	15.58	35	2.2
<i>S. equorum</i>	2.61	4	1.5
<i>S. chromogenes</i>	4.39	5	1.1
<i>S. succinus</i>	0.92	1	1.1
<i>S. xylosus</i>	18.38	17	0.9
<i>S. haemolyticus</i>	5.11	4	0.8

4. Discussion

Cow-based calf rearing systems are gaining increasing attention as consumers demand more natural husbandry practices in dairy farming. However, the direct and prolonged udder contact associated with these systems raises concerns about transmission of pathogens between cows and calves. Understanding the dynamics of pathogen transmission is essential for farmers and veterinarians to develop appropriate health management strategies. Therefore, the objective of this cross-sectional study was to investigate the transmission of mastitis-associated pathogens between dairy cows and suckling calves across multiple farms that practice cow-based calf rearing.

NaS/*Mammaliicoccus* spp. and *Corynebacterium* spp. were the most frequently detected pathogens in the milk samples of the dams and foster cows. NaS/*Mammaliicoccus* spp., especially *M. sciuri*, *S. xylosus*, and *S. equorum*, were also the dominant species in the calf's saliva. Concerning the milk samples, NaS/*Mammaliicoccus* spp. and *Corynebacterium* spp. are among the most frequently detected pathogens in other studies conducted on both conventional and organic dairy farms during early lactation. For example, Nitz et al. [10] detected 7.1% NaS and 1.4% *Corynebacterium* spp. in heifers on conventional dairy farms 17 + 3 days after calving. Mütze et al. [11] investigated the differences between antibiotic dry-off treatment with and without additional internal teat sealant on 12 dairy farms. Therefore, they collected milk samples at different time points, including early lactation (between days 6 and 14 after calving and between days 35 and 56). Between days 35 and 56 of lactation, *Corynebacterium* spp. was detected in 35 quarters and NaS in 16 quarters of a total of 244 udder quarters sampled in the group with internal teat

sealant, and *Corynebacterium* spp. in 53 quarters and NaS in 23 quarters in the group without sealant. Although pathogen prevalences in that study could have been influenced by dry-off treatment, NaS and *Corynebacterium* spp. were nonetheless the most frequently detected pathogens. On organic dairy farms, Kiesner et al. [12] also reported substantial prevalences of these pathogens within 5 to 12 days in milk, with 33.2% detected NaS and 24.4% *Corynebacterium* spp. Although these figures were calculated at the cow level and are therefore not directly comparable to the quarter-level prevalences reported in the present study, the overall pattern of NaS and *Corynebacterium* spp. as the dominant pathogens is consistent.

NaS comprises a heterogeneous group of different pathogens [13] that are primarily detected in connection with subclinical mastitis [14]. However, the representatives of this group exhibit different characteristics, and some of them (e.g., *S. chromogenes*, *S. haemolyticus* or *M. sciuri*) can also cause clinical mastitis [14,15]. NaS can be found on teat skin, teat canal but also in the environment of the animals like bedding or floor, which calves and cows in the present study share. Consequently, strain concordances between cow and calf samples may result either from direct transmission or from independent colonization via the shared environment. In the present study, *M. sciuri* strains were matched not only between individual cow–calf pairs but also between different calves of the same foster cow and between foster cows within a group. Notably, the strain-level matches recorded as cow–calf concordances in the present dataset were part of this broader within-group overlap, as the same strain was simultaneously detected in multiple foster cows and multiple calves housed together. Although the observed-to-expected ratio of 2.2 indicates that matches occurred more frequently than expected by chance, it was lower than the ratio observed for *S. aureus* (2.9). In addition, the groupwide distribution pattern suggests environmental circulation rather than a direct cow-to-calf transmission during suckling. This is further supported by the prevalence data from the present study. *M. sciuri* and *S. xylosus* showed the highest prevalence in calf saliva (30.5% and 33.3%, respectively), which significantly exceeded their prevalence in cow's milk (8.9% and 9.7%, respectively). This resulted in a saliva-to-milk ratio of 3.4 for both pathogens. This suggests that these species colonize the oral cavity of calves largely independently of milk, likely via the shared environment. Other NaS like *S. chromogenes*, *S. equorum*, *S. haemolyticus*, *S. succinus* and *S. vitulinus* also showed species-level matches (5, 4, 4, 1 and 1, respectively) but no strain-level matches, suggesting that these species are widely distributed in the herd environment, with many distinct strains circulating. Their detection in both milk and calf saliva further indicates that these NaS species are capable of colonizing both ecological niches.

The prevalence of *S. aureus* in the milk samples was slightly higher in the present study than in the study of Kiesner et al. [12], who detected 6.8% of cows positive for *S. aureus* between days 5 to 12 in milk. In another study on organic dairy farms, *S. aureus* was found in 10.7% of quarter milk samples [16]. However, these samples were not taken during early lactation, but at random times from randomly selected cows. *S. aureus* was also detected in the saliva samples of some calves, and there were both species and strain matches between milk samples from cows and saliva samples from their calves. *S. aureus* is a contagious pathogen with transmission normally taking place during milking via infected milk. A previous study using RAPD-PCR demonstrated that identical *S. aureus* strains can be detected in foster cows and their associated calves [3]. This highlights the contagious nature of this pathogen. Consistent with these findings, identical *S. aureus* strains were detected in the present study in 3 foster cow–calf pairs across two farms. On one farm, the same strain was shared between a single foster cow and two of her corresponding calves, and on a second farm between one foster cow and one calf. These observations support the assumption that calves can be exposed to *S. aureus* during suckling from

infected teats. This is supported by the observed-to-expected ratio of 2.9 for *S. aureus*, which indicates that *S. aureus* matches occurred almost three times more frequently than would be expected only by chance. However, the limited overall strain concordance between pathogens isolated from cow milk and calf saliva indicates that oral exposure does not necessarily result in stable colonization of the calf's salivary microbiome. This is consistent with the low saliva-to-milk ratio of 0.3 for *S. aureus*, which indicated that although *S. aureus* is relatively frequently present in cow milk (9.3%), it was detected less frequently in calf saliva (3.2%). While identical strains of *M. sciuri* were detected across multiple foster cows and calves within the same group which points towards environmental origin and within-group circulation as the primary source, *S. aureus* strain matches were restricted to individual foster cows and their own calves, consistent with direct transmission via infected milk during suckling, reflecting the well-established role of *S. aureus* as an intramammary pathogen.

P. multocida was also detected in the milk samples. This pathogen is involved in respiratory infections in calves and is therefore regularly detected in the upper respiratory tract of calves, e.g., via deep nasal swabs [17,18]. However, nowadays, in dairy farms without calf-cow-contact, it plays a minor role as a cause of mastitis [4]—in contrast to beef cow systems, where *P. multocida* is a common pathogen causing mastitis, potentially because suckling contact is common [5]. In the study by Köllmann et al. [3], identical strains of *P. multocida* were found in foster cows and their calves using RAPD-PCR. Calves with respiratory infections can carry *P. multocida* in high numbers in the nasopharynx from where it can also spread to the oropharynx, allowing it to remain temporarily in the oral cavity. During suckling, these bacteria can be mechanically transmitted to the udder of cows. In the present study, four species-level concordances were observed between cow-calf pairs, against an expected frequency of only 0.19 based on the individual prevalences in each sample type—resulting in an observed-to-expected ratio of 21.1, the highest of all pathogens examined. This markedly non-random co-occurrence suggests that the shared detection of *P. multocida* in cow milk and calf saliva is unlikely to be coincidental and may reflect a transmission-related association, consistent with the direction of transmission proposed above. For *P. multocida*, strain typing to confirm whether the isolates were clonally identical could not be performed in the present study because the isolates were no longer culturable at the time of analysis. To reduce the risk of *P. multocida* transmission, respiratory infections in calves should be prevented by ensuring sufficient colostrum supply, good hygiene, proper ventilation, and minimizing stress.

Overall, only few strain concordances were observed between milk and calf saliva samples. Possible explanations for this include a combination of biological and methodological factors. First of all, transmission is probably dependent on the bacterial load (“mass” effect). If there is a high bacterial count in the oral cavity (e.g., in the case of a respiratory infection, *P. multocida*), sufficient bacteria could enter the milk through suckling. The same applies, of course, in the opposite direction, from the milk (in the case of *S. aureus*, for example) into the saliva. The saliva samples tested positive for *P. multocida* were collected in November and May. The milk samples tested positive for *P. multocida* were collected in November, March, July, and August. All species matches for *P. multocida* were found in November. Since calves are more susceptible to respiratory infections in winter, the presumably high bacterial load at the time of sampling may be responsible for the matches.

Another explanation could be that pathogens can theoretically localize or be “trapped” in the tonsils, which may limit their detectability in saliva. This concept is supported by Daleel and Frost [19], who found mastitis-associated pathogens like *Sc. dysgalactiae* (7.3%) and *Sc. uberis* (15.8%), but also *S. aureus* (7%), in the tonsils of healthy slaughtered cattle and calves. Furthermore, *Mycoplasma (M.) bovis* has previously been detected in the tonsils

of euthanized calves following ingestion of milk replacer mixed with an aliquot of *M. bovis* [20], suggesting that certain pathogens may persist in tissue-associated niches rather than being detectable in free saliva.

Furthermore, both calf saliva and raw milk exhibit antimicrobial activity, particularly due to lysozyme, which is bactericidal—especially against Gram-positive organisms—and can damage bacterial cell walls [21,22]. As a result, milk-associated bacteria could be reduced or eliminated shortly after entering the oral cavity, as could bacteria from the oral cavity in the milk. In addition, there is competition from the resident oral flora, which can quickly reduce or eliminate temporarily ingested bacteria.

Finally, it should be noted that both milk samples and oral cavity samples were taken at a single time point—the end of the suckling period. With this approach, it is possible to capture the microbiological status after the prolonged cow–calf contact and thereby maximize the opportunity to detect transmission. However, this cross-sectional approach provides only a snapshot of a dynamic process, temporal variations in prevalence of pathogens may have been missed, and rare or short-lived strains may have been missed. Collectively, these factors could explain the limited concordance.

A further methodological aspect concerns sampling itself. While the suckling calf is naturally exposed to the teat-canal and teat-skin flora as well, these microorganisms were not the subject of the present investigation. Aseptic milk sampling was deliberately chosen because the focus of this study was on intramammary mastitis-associated pathogens rather than on microorganisms residing in the teat canal or environment-associated bacteria present on the teat skin. Discarding the first streams of milk and disinfecting the teat apex therefore ensured that detected pathogens reflected true intramammary infection rather than transient contamination.

All strain concordances were found in foster cow–calf pairs. This finding was statistically significant (Fisher’s exact test, $p = 0.012$), though it should be interpreted in the context of the unequal group sizes in the present dataset (391 foster cow–calf pairs vs. 181 dam–calf pairs), as well as the distribution of concordances across farms: nine of the 12 strain matches originated from a single farm, where three foster cows and three calves housed in a shared group all carried the same *M. sciuri* strain. Several biological and management-related factors may contribute to this pattern. Foster cows suckle multiple calves, either sequentially or simultaneously, resulting in a greater number of udder contacts per cow compared to dams. This increases the opportunity for bidirectional pathogen transfer during suckling. Furthermore, in foster cow systems, calves are often housed in shared groups and may have contact with multiple foster cows, facilitating within-group pathogen circulation. This transmission pathway may become particularly relevant on farms using so-called “hop-on” systems, in which foster cows return to the regular milking herd after weaning. In such cases, cows re-enter the milking process, increasing the risk of pathogen transmission (especially *S. aureus* and *P. multocida*) via the conventional milking-associated route. It should be noted that farms on which strain concordances were detected were characterized by large group sizes, with a high number of calves per foster cow, resulting in a large number of foster cow–calf pairs.

Study Limitations

When interpreting the results, it should be considered that sampling differed slightly between farms. On farms where the cows (either dams or foster cows) were also milked, samples were collected in the milking parlor immediately before milking. On the other farms, milk samples were taken at various times throughout the day. These differences in the timing of sampling may have influenced the detection of bacteria and should be taken into account.

Strain-level typing was done using RAPD-PCR, which offers a rapid and cost-effective approach to assess whether isolates are clonally related. While more precise and reproducible methods such as pulsed-field gel electrophoresis (PFGE) or whole-genome sequencing (WGS) exist, RAPD-PCR was sufficient for the purposes of this study, as unrelated isolates could be clearly distinguished. Within a single farm, isolates showed high genetic diversity, with most pairwise comparisons differing by more than five bands. This suggests that RAPD-PCR had enough discriminatory power to distinguish unrelated strains under the conditions of this study. Matching banding patterns were exclusively observed within cow–calf pairs, further supporting the validity of the approach. In addition, all isolates were analyzed in parallel under identical experimental conditions to minimize variability between runs. RAPD-PCR products that produced identical patterns in the first electrophoresis were then loaded side by side onto a second gel; isolates were regarded as the same strain only when no band differences were detectable.

A further potential limitation of this study is the voluntary participation of farms, which may introduce a selection bias. However, in the present population, the implementation of cow-based rearing systems is predominantly motivated by ethical and animal-welfare considerations rather than by management optimization, and even among organic farms only a minority currently adopt such systems. As this motivation is not management-driven, participating farms are unlikely to be systematically selected towards superior husbandry management. This mitigates the concern that the observed results reflect above-average herd management rather than the characteristics of the rearing system itself.

5. Conclusions

Our results indicate strain concordance compatible with transmission of mastitis-causing pathogens between calves and cows. This was particularly evident for *S. aureus*. Overall, however, relatively few strain matches were found between calf saliva and cow milk, which can be attributed to both biological and methodological limitations. For *P. multocida*, the strongest non-random co-occurrence was detected with an observed/expected ratio of 21.1—the highest of all pathogens examined. Species matches suggest transmission from the calves' nasopharynx to the udder during suckling, particularly in cases of respiratory infections, where *P. multocida* can also spread to the oropharynx and temporarily colonize the oral cavity. Strain typing could not be performed for this pathogen because the isolates were no longer cultivable. Although *M. sciuri* showed many species and some strain matches, these were distributed within the group context (multiple cows and calves with identical strains), suggesting a common environmental source rather than direct transmission. All strain matches were detected exclusively in foster cow–calf pairs, which was statistically significant (Fisher's exact test, $p = 0.012$). Measures to reduce *P. multocida* and *S. aureus* transmission are particularly relevant in foster cow systems.

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on 13 July 2020. According to the local government, an application for animal testing was not necessary. The International Guiding Principles for Biomedical Research Involving Animals (1985) were followed.

Informed Consent Statement: Informed consent was obtained from all participating farm owners prior to sample collection.

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References

1. Placzek, M.; Christoph-Schulz, I.; Barth, K. Public attitude towards cow-calf separation and other common practices of calf rearing in dairy farming—A review. *Org. Agric.* **2021**, *11*, 41–50. [CrossRef]
2. Schmenger, A.; Krömker, V. Characterization, cure rates and associated risks of clinical mastitis in Northern Germany. *Vet. Sci.* **2020**, *7*, 170. [CrossRef] [PubMed]
3. Köllmann, K.; Wente, N.; Zhang, Y.; Krömker, V. Investigations on transfer of pathogens between foster cows and calves during the suckling period. *Animals* **2021**, *11*, 2738. [CrossRef]
4. German Veterinary Medical Association (GVA). Zur Prävalenz von Mastitisserregern in Milchproben in Deutschland—Update 2022. In Proceedings of the Tagung der AG Eutergesundheit der DVG, Schwäbisch Gmünd, Germany, 18–19 March 2024. Available online: https://www.dvg.de/wp-content/uploads/2024/04/24-03-19_AG-Euter_Praevalenz-Mastitisserreger2022.pdf (accessed on 3 February 2026).
5. Vollenweider, A.; Corti, S.; Hochreutener, M.; Biner, B.; Stephan, R.; Bleul, U. Mastitis pathogens and antibiotic resistance in beef cows in Switzerland. *Schweiz. Arch. Tierheilkd.* **2023**, *165*, 39–51. [CrossRef] [PubMed]
6. German Veterinary Medical Association (GVA). *Leitlinien zur Labordiagnostik der Mastitis—Probenahme und Mikrobiologische Untersuchung*, 3rd ed.; Verlag der Deutschen Veterinärmedizinischen Gesellschaft: Gießen, Germany, 2018.
7. Heeschen, W.; Reichmuth, J.; Tolle, A. Die Konservierung von Milchproben zur bakteriologischen, zytologischen und hemmstoffbiologischen Untersuchung. *Milchwissenschaft* **1969**, *24*, 729–734.
8. Gillespie, B.E.; Jayarao, B.M.; Pankey, J.W.; Oliver, S.P. Subtyping of *Streptococcus dysgalactiae* and *Streptococcus uberis* isolated from bovine mammary secretions by DNA fingerprinting. *J. Vet. Med.* **1998**, *45*, 585–593. [CrossRef]
9. Damiani, G.; Telecco, S.; Comincini, S.; Sironi, M.; Carretto, E.; Marone, P. Comparison of an improved RAPD fingerprinting with different typing methods for discriminating clinical isolates of *Staphylococcus* spp. *Eur. J. Epidemiol.* **1996**, *12*, 163–169. [CrossRef] [PubMed]
10. Nitz, J.; Wente, N.; Zhang, Y.; Klocke, D.; tho Seeth, M.; Krömker, V. Dry period or early lactation—Time of onset and associated risk factors for intramammary infections in dairy cows. *Pathogens* **2021**, *10*, 224. [CrossRef] [PubMed]
11. Mütze, K.; Wolter, W.; Failing, K.; Kloppert, B.; Bernhardt, H.; Zschöck, M. The effect of dry cow antibiotic with and without an internal teat sealant on udder health during the first 100 d of lactation: A field study with matched pairs. *J. Dairy Res.* **2012**, *79*, 477–484. [CrossRef] [PubMed]
12. Kiesner, K.; Wente, N.; Volling, O.; Krömker, V. Selection of cows for treatment at dry-off on organic dairy farms. *J. Dairy Res.* **2016**, *83*, 468–475. [CrossRef] [PubMed]
13. Vanderhaeghen, W.; Piepers, S.; Leroy, F.; Van Coillie, E.; Haesebrouck, F.; De Vlieghe, S. Identification, typing, ecology and epidemiology of coagulase negative staphylococci associated with ruminants. *Vet. J.* **2015**, *203*, 44–51. [CrossRef] [PubMed]
14. Condas, L.A.Z.; De Buck, J.; Nobrega, D.B.; Carson, D.A.; Roy, J.P.; Keefe, G.P.; DeVries, T.J.; Middleton, J.R.; Dufour, S.; Barkema, H.W. Distribution of non-*aureus* staphylococci species in udder quarters with low and high somatic cell count, and clinical mastitis. *J. Dairy Sci.* **2017**, *100*, 5613–5627. [CrossRef] [PubMed]
15. Addis, M.F.; Locatelli, C.; Penati, M.; Poli, S.F.; Monistero, V.; Zingale, L.; Rota, N.; Gusmara, C.; Piccinini, R.; Moroni, P.; et al. Non-*aureus* staphylococci and mammaliococci isolated from bovine milk in Italian dairy farms: A retrospective investigation. *Vet. Res. Commun.* **2024**, *48*, 547–554. [CrossRef] [PubMed]

16. Barth, K.; Krömker, V.; Aulrich, K. *Einstreumaterialien und-Management–Ihre Bedeutung für die Entwicklung von Mastitiserregern und das Infektionsgeschehen in der Ökologischen Milchviehhaltung*; Abschlussbericht FKZ 2808OE196 & 2809OE012; Thünen-Institut für Ökologischen Landbau: Westerau, Germany, 2011. Available online: https://orgprints.org/id/eprint/19933/1/19933-08OE196-09OE012-vti-fh_hannover-barth-kroemker-2011-einstreumaterialien_milchvieh.pdf (accessed on 17 February 2026).
17. Lachowicz-Wolak, A.; Klimowicz-Bodys, M.D.; Płoneczka-Janeczko, K.; Bykowsy, M.; Siedlecka, M.; Cinciała, J.; Rypuła, K. The prevalence, coexistence, and correlations between seven pathogens detected by a PCR method from south-western Poland dairy cattle suffering from bovine respiratory disease. *Microorganisms* **2022**, *10*, 1487. [[CrossRef](#)] [[PubMed](#)]
18. Denholm, K.; Evans, N.P.; Baxter-Smith, K.; Burr, P. Retrospective study of the relative frequency of cattle respiratory disease pathogens in clinical laboratory samples submitted by UK veterinary practices. *Vet. Rec.* **2024**, *195*, e4434. [[CrossRef](#)] [[PubMed](#)]
19. Daleel, E.E.; Frost, A.J. Some observations on the bacterial flora of the bovine tonsil. *Br. Vet. J.* **1967**, *123*, 232–236. [[CrossRef](#)] [[PubMed](#)]
20. Maunsell, F.; Brown, M.B.; Powe, J.; Ivey, J.; Woolard, M.; Love, W.; Simecka, J.W. Oral inoculation of young dairy calves with *Mycoplasma bovis* results in colonization of tonsils, development of otitis media and local immunity. *PLoS ONE* **2012**, *7*, e44523. [[CrossRef](#)] [[PubMed](#)]
21. Gilmutdinov, R.Y.; Spiridonov, G.N.; Shalamova, G.G.; Aleksandrova, N.M.; Shamaev, A.G. Biotic and abiotic factors of bactericidal properties variability of ruminant saliva. *BIO Web Conf.* **2020**, *27*, 00051. [[CrossRef](#)]
22. Krömker, V. *Kurzes Lehrbuch Milchkunde und Milchhygiene*, 1st ed.; Verlag Parey: Stuttgart, Germany, 2006; p. 205.

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