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Management and housing factors associated with paratuberculosis-positive herds in small structured alpine cattle husbandry

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ABSTRACT

Paratuberculosis (Johnes̓ disease) is a world-wide cattle disease caused by *Mycobacterium avium* subsp. *paratuberculosis* (MAP), associated with substantial economic losses. Purchase of subclinically infected animals or contact with animals and equipment of infected farms are known risk factors for disease transmission among herds. The aim of the present study was to identify specific management factors in Austria that triggered a MAP-positive herd status and to evaluate known risk factors for the transmission in cattle in small structured alpine agricultural systems. The agriculture in the Austrian province of Tyrol is characterized by smallholder structures, including shared alpine pastures and traditional barn management techniques. The data from an extensive survey with 50 questions in 2013/2014 and the development of the MAP herd status of 5592 cattle farms by taking feces and blood samples were examined and statistically evaluated. MAP herd status was determined by combining the results of boot swab samples, manure samples, pooled and individual feces samples as well as serological antibody testing by ELISA. The statistical analysis (odds ratio; OR) showed that the use of milk replacers for calf feeding ($p = 0.047$, OR=0.472) and the use of straw as bedding material for cows ($p = 0.032$, OR=0.625) were associated with a decreased chance of being a MAP-positive herd. Further, housing cows in deep litter systems ($p = 0.028$, OR=2.232), the presence of slurry channels ($p = 0.028$, OR=1.411) and the use of solid manure in young cattle ($p = 0.041$, OR=1.744) were associated with an increased OR for being MAP-positive. Surprisingly, sharing of lowland pastures ($p = 0.564$, OR=1.080), alpine pastures ($p = 0.419$, OR=1.143) or farm equipment ($p = 0.733$, OR=0.963) and farm size ($p = 0.425$) had no significant influence on the MAP herd status. The identified differences compared with previously published results in respect of MAP spread in cattle might be attributed to the traditional agricultural structures, including small family-based farms and common pasture during summer in alpine regions. Results of this study contribute to the understanding of the spread of MAP in cattle farming in alpine regions.

1. Introduction

Paratuberculosis, or Johne's disease (JD), is caused by *Mycobacterium avium* ssp. paratuberculosis, MAP (McAloon et al., 2019). MAP infection becomes chronic in cattle, is transmitted mainly via the fecal-oral route within the first months of life and has high environmental tenacity (Rowe and Grant, 2006). A period of at least two years of latency usually follows early exposure (Sweeney, 2011). In this early stage of infection MAP is usually not detectable by antibodies or antigen tests and infected animals do not show any symptoms of JD. This makes it difficult to detect animals at an early stage of infection (Sweeney, 2011). Only after about two years post-infection does fecal shedding of MAP occur and with it the production of specific antibodies. In most cases clinical symptoms of JD are still not apparent (Sweeney, 2011). The onset of characteristic clinical signs, such as watery diarrhea and weight loss, despite normal appetite, are quite variable (McAloon et al., 2016,

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2017). JD is untreatable and no efficient vaccines are currently available for cattle (Lisle, 2010; Arsenault et al., 2014). Consequently, sanitation of affected farms is based on control programs such as the "test, cull and control" strategy, combined with hygiene and management measures to prevent new infections (Sweeney et al., 2012; Verdugo et al., 2015; Imada et al., 2023). Cattle tested positive for the disease must be separated and removed from the herd. Continuous testing of animals is used to limit further spread within herds.

MAP infections in cattle are emerging in most regions of the world. In Europe, up to 68% of cattle herds have been reported to be MAP-positive in some countries (Nielsen and Toft, 2009; Eisenberg et al., 2022), but estimation of the prevalence is difficult, due to the low sensitivity of laboratory tests available (Barkema et al., 2018). In Austria, clinical JD is a notifiable disease, necessitating culling of infected animals (Khol et al., 2019). The herd seroprevalence in Austria was earlier estimated at 19.1% (Baumgartner et al., 2005). The Austrian province Tyrol, the region of study, is characterized by its traditional alpine agriculture, the use of small barns with an average herd size of 24 cattle and mainly common alpine pasturing of different age groups and herds during the summer months. In a more recent survey, a MAP herd prevalence of 8% (Köchler et al., 2017), was detected in Tyrol by using boot swabs for sample collection.

In 2013, the Tyrolean Animal Health Service launched a voluntary control program for paratuberculosis in cattle by screening 4718 farms by boot swab sampling, which has been proven to be effective in detecting MAP-positive herds with a sensitivity of up to 90.6% when applied repeatedly (Eisenberg et al., 2013; Wolf et al., 2016). Participating farms had to complete a survey with questions about farm structure, management practices, housing concepts and livestock rearing. After the first investigation participating farms were tested for MAP at two-year intervals, based on boot swab sampling, followed by single animal testing in positive herds (Khol et al., 2019).

The aim of this study was to identify risk factors associated with a positive MAP herd status based on the 2013 management data and the consecutive MAP testing. Thereby, this should identify particular risk factors for the transmission of MAP in cattle for these small structured alpine agricultural systems. Close attention was paid to some typical characteristics of alpine farming that are passed down from generation to generation over hundreds of years, and which might impact the transmission of MAP.

2. Materials and methods

This study refers to a database of a total of 5592 cattle farms that were examined for MAP between 2013 and 2018 in the Austrian province of Tyrol. The screening was part of the control program for paratuberculosis in cattle enforced by the Tyrolean Animal Health Service. In the course of this voluntary control program participating farms are tested for MAP by analyzing boot swab samples at two-year intervals, followed by single animal testing (feces and serum) in positive farms (Khol et al., 2019; Sodoma et al., 2021). At the first round of boot swab sampling in 2013, herd and management data were additionally collected from participating farms. We analyzed these data to identify possible associations between farm structures and management practices and farm MAP status.

2.1. Farms enrolled

Altogether there are about 12,500 cattle farms in Tyrol, of which 5712 farms (effective 2018) were participating in the Tyrolean Animal Health Service. In 2013/2014, a total of 4718 of these farms with unknown MAP status were sampled. In 2015, 239 farms with positive and 155 farms with negative status from the first round were resampled. Furthermore, seven new farms with unknown status were added to the program. The third round in 2016/2017 included 3248 farms from 2013/2014 with known status (1.34% positive), 260 farms from 2015 with known status (10.64% positive) and 760 new farms with unknown status. In 2017/2018, 157 farms from 2013/2014, 2015 and 2016/2017 were resampled (altogether 47.13% positive) and 107 farms with unknown status were added to the sampling program (Fig. 1). Six farms (0.1%) were examined four times, 350 (6.3%) farms three times, 3341 (59.7%) farms twice (overall-status 3.23% positive) and the remaining 1895 (33.9%) farms were examined only once (overall-status 3.32% positive) in course of the program.

The mean farm size of participating farms in 2013 was 24.35 cattle per farm (min two, max 269; median 19). The majority of the animals were Austrian Simmental (41.1%) and Brown Swiss (24.6%) breeds. This represents the typical structure of the family-based agricultural structure in the Austrian alpine region. Altogether, about 55% of the Tyrolean cattle holders and 61% of the cattle population were enrolled in the study (Khol et al., 2019).

2.2. Sampling procedures

All animals of boot swab positive farms in 2012/2014 and 2016/2017 were sampled by individual fecal samples (Khol et al., 2019). All animals of the 239 farms in 2015 and of the 17 farms in 2017/2018 were tested one more time, respectively. However, it must be mentioned that a small number of farms could not be sampled strictly according to this procedure and were only examined in the following round of examinations. Feces was collected directly from the rectum of the animals as described by Donat et al. (2016) and blood samples were taken for antibody testing by ELISA (enzyme linked immunosorbent essay).

2.3. Collection of boot swab samples

Boot swab samples were taken in accordance with the method described by Donat et al. (2016). The person conducting the farm samplings wore specific single use overshoes with an absorbing material on the sole (socks). Wearing these boot swabs the sampling person walked in a meandering manner through farm walkways (loose housing systems) or slurry channels (tethered housing), so that the absorbent socks were soaked with approximately 50 g of feces and the entire relevant barn area had been traversed (Eisenberg et al., 2013). After collection, the boot swabs were packed into sterile plastic bags, cooled, and sent to the Institute for Bacteriology at the University of Veterinary Medicine Vienna (samples of 2013/2014 and 2015) or the National Reference Laboratory for Paratuberculosis of the Austrian Agency for Food Safety, AGES Linz, Austria (samples of 2016/2017 and 2018). In farms with less than five eligible cows, pooled individual fecal samples were collected rectally from animals with a minimum age of 24 months instead of collecting boot swab samples.

Additionally, the MAP status was obtained from follow-up samplings in the course of the MAP program consisting of further boot swabs, pooled and individual animal fecal samples and serological antibody titers (Fig. 1). All samples were collected by veterinarians in a standardized manner.

2.4. Manure sampling

In the course of this study the sensitivity of combining manure samples and boot swabs as described by (Donat et al., 2016) to boot swab sampling alone in family-based agricultural structures were also evaluated. 138 farms during 2017/2018 were tested by boot swaps and additionally by manure sampling. The majority of these farms (106) were tested for MAP for the first time.

For the collection of manure samples a mechanical extension arm was used to evacuate approximately 100 g of manure from at least 10 cm below the surface of the liquid manure pit; this sample was then transferred into a plastic cup (Donat et al., 2016). If there was only a manure heap, approximately 100 g of manure was taken from at least three different positions in the heap and pooled together in one plastic

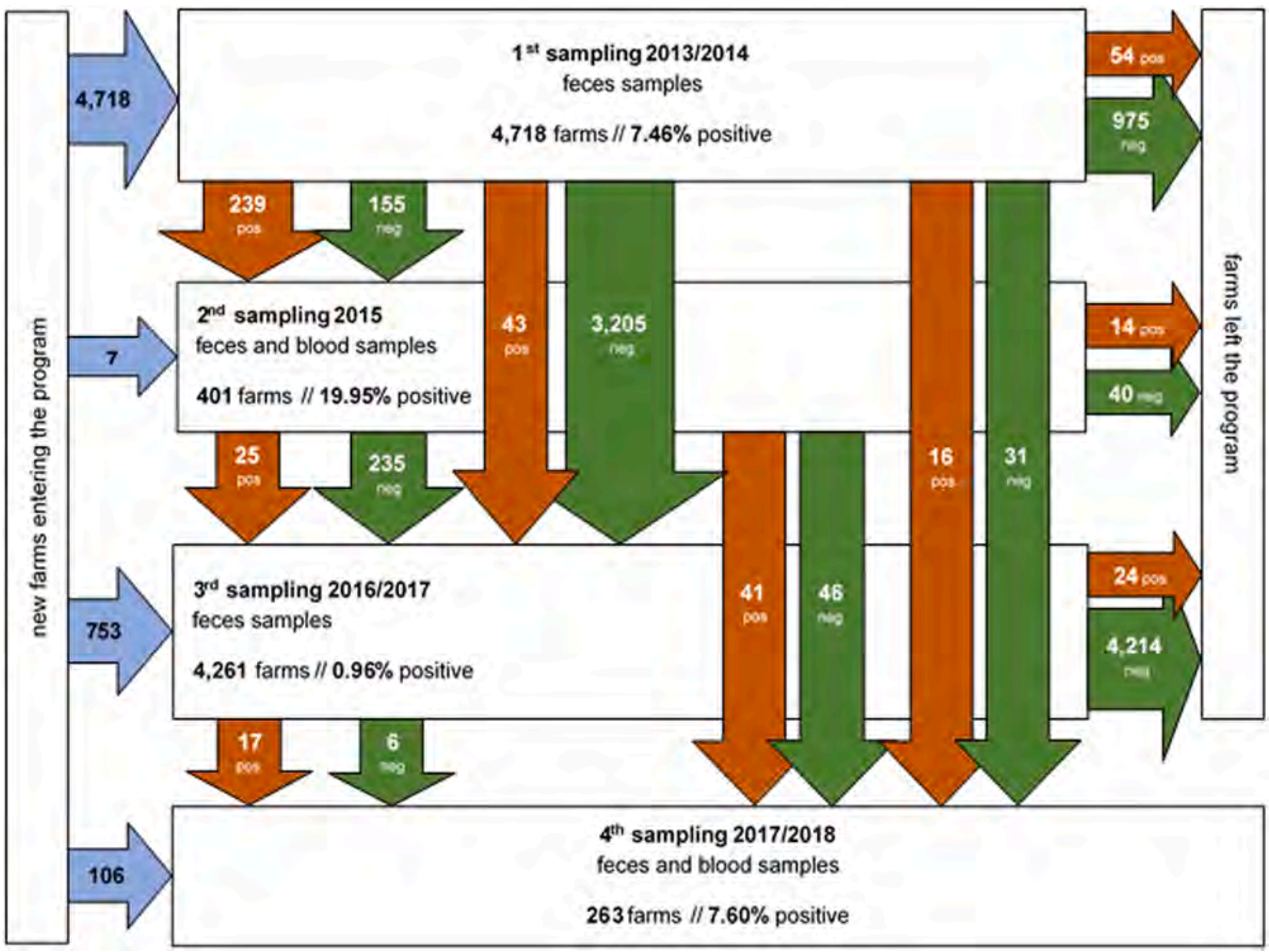

Fig. 1. Farms and samplings enrolled in the study. The numbers in arrows show the count of farms. The white boxes represent the four different examinations with the evaluated sampling matrix.

cup.

2.5. Blood sampling

Blood samples were collected with the Vacutainer® system (Greiner Bio-One International GmbH, Kremsmünster, Austria) from the coccygeal vein by using tubes with Z serum clot activator.

2.6. MAP detection in feces by culture and PCR

The boot swab samples were examined for MAP, according to the same procedure in the two aforementioned laboratories, and as described by (Gschaider et al., 2021; Köchler et al., 2017; Sodoma et al., 2021) using a method combining bacterial culture and PCR. In the laboratories boot swab samples were transferred to Stomacher® bags (Seward Ltd., Worthing, UK) and homogenized (LB 400 circulator, VWR International LLC, Vienna, Austria) for 60 s. Following homogenization, 50 ml of phosphate buffered saline (PBS) were added. Subsequently samples were centrifuged at 3000 g for 15 min and the supernatant discharged. Three grams of the remaining manure were resuspended with 30 ml of 0.75% hexadecylpyridinium chloride (HPC, Sigma Aldrich Inc., St. Louis, USA), shaken for 60 min, followed by five minutes of sedimentation. Afterwards, 15 ml of the supernatant were transferred into a sterile tube and incubated in the dark for 48 h at room temperature. The samples were then centrifuged again at 3000 g for 15 min for decontamination. The supernatant was discharged and the pellets mixed with 1 ml of 0.75% HPC. For bacterial culture, 0.2 ml of each sample were transferred into four tubes of Herrolds egg yolk medium (HEYM), which was prepared in-house and contained 2 mg of Mycobactin J (Pourquier®, IDEXX GmbH, Ludwigsburg, Germany or Becton, Dickinson and Company, Franklin Lakes, USA) per litre. All prepared tubes were incubated at 37 °C and checked for growth of MAP every week for a total of 12 weeks. After four weeks of incubation, the contents of one of the four tubes per sample was rinsed with 200 µl PBS or 400 µl distilled water and the fluid used for real-time PCR to detect MAP. For extraction of the DNA the QIAmp DNA Stool Mini Kit (Qiagen N.V., Venlo, Netherlands) was used. The MAP-specific sequence element was amplified by using the VetMAX™ MAP real-time PCR screening kit (Fisher Scientific Austria GmbH, Vienna, Austria), again following the manufacturer's instructions using a C1000 Touch Thermal Cycler (Bio-Rad Laboratories GmbH, Vienna, Austria) for amplification (Gschaider et al., 2021; Köchler et al., 2017). A different method used IndiMag® Pathogen Kit (Indical Bioscience GmbH, Leipzig, Germany) for extraction in KingFisher Extracitonrobot™ (Thermo Fisher Scientific, Waltham, USA) followed by real-time PCR with bactotype® MAP-PCR-Kit (Qiagen GmbH, Hilden, Germany) (Sodoma et al., 2021). If growth of MAP occurred on one of the three remaining tubes, colonies were sampled by PCR as described above for confirmation of the presence of MAP. Culture tubes not showing any MAP colonies after 12 weeks of incubation were rated as negative.

Pooled and individual fecal samples, as well as manure samples from the manure storage containers were tested for MAP as described above for boot swab samples.

2.7. MAP detection in blood samples by ELISA

Blood samples from 2015 (Gschaider et al., 2021) were sent to the University for Veterinary Medicine Vienna and tested for MAP with the ID-Screen® Paratuberculosis Indirect ELISA (IDvet - Innovative Diagnostics, Grabels, France). Positive and suspicious samples were sent to the National Reference Laboratory for Paratuberculosis at the AGES Institute for Veterinary Disease Control Linz for confirmation. They were tested with the IDEXX® Paratuberculosis Screening Antibody Test (IDEXX GmbH, Ludwigsburg, Germany). Blood samples from 2016 to 2018 were sent to the National Reference Laboratory for Paratuberculosis of the Austrian Agency for Food Safety (AGES Linz, Austria) only and tested for specific antigens using the IDEXX® Paratuberculosis Screening Antibody Test (IDEXX GmbH, Ludwigsburg, Germany).

2.8. Assigning MAP herd status

Overall, four rounds of feces sampling from 2013 to 2018, with additional samplings between these dates (Fig. 1), were performed in the course of the MAP control program and considered within the present study. In the course of the samplings performed within the Tyrolean MAP-program described above, the following results were obtained: The first round of fecal samples performed in 2013/2014 revealed 7.46% MAP-positive farms. In 2015, 19.95% of 401 resampled farms with an known status and increased risk for being MAP-positive were considered to be MAP-positive (Köchler et al., 2017). The third round of 2016/2017, 0.96% cattle herds were positive (Khol et al., 2019). Examination results in 2017/2018 revealed 7.60% of 263 tested farms to be MAP-positive (Fig. 1).

2.9. Definition of MAP-positive farms

In the course of this study a farm was defined as MAP-positive if any investigation from 2013 to 2018 revealed a MAP-positive result. This includes all methods used, both antibody tests (blood ELISA) and direct pathogen detection (fecal culture and PCR). Thereby a total of 7.2% (405) of all tested farms from 2013 until 2018 were considered MAP-positive. Conversely, 5187 (92.8%) farms were tested negative in all investigations.

The six farms (0.10%) that were present in all four runs were all positive, following this definition. From the farms that were sampled three times (350 farms, 6.26%) 65.14% were positive, from farms that were sampled twice (3341 farms, 59.74%) 3.23% were positive, and of farms that were sampled once (1895 farms, 33.88%) 3.32% showed positive results, respectively. The origin of the positive farms in relation to the testing procedures can be seen in Fig. 1.

2.10. Collection of herd data

In the course of the first investigation of the Tyrolean MAP program in 2013 a farm survey was conducted by the local veterinarian before samples were collected. The survey consisted of 50 questions and was completed by all farms of the first sampling in 2013/2014 (4718). 78.19% (3689) of these farms completed at least two rounds of MAP testing in course of the program. All variables of the questionnaire, except herd size, were dichotomous (see supplemental material). The survey data are divided into six sections:

First section: *structural data* with eight questions aimed at describing the farm structure and size. Second section: *housing of cows*, third section: *housing of young cattle*. Both sections dealt with different housing systems, consisting of 10 questions each (Tables 1 and 2). Fourth section: *livestock rearing* dealt with the rearing regime of calves and consisted of nine different questions (Table 3).

Fifth section: *management at the farm*, like using specific cow pens or breeding technics (8 questions, Table 4).

Sixth section: *management off the farm*, such as sharing of pastures or equipment with other farms related to MAP transmission were requested (5 questions, Table 5).

Table 1
Impact of housing of cows on being assigned MAP-positive herd status by using multivariable analysis.

Housing of cows	F	p	OR	Confidence interval
tethered stall	82.5%	0.372	1.446	0.644–3.245
open cowsheds	18.1%	0.676	0.822	0.328–2.060
Cubicles	13.5%	0.372	1.398	0.670–2.919
housing in deep litter systems	03.6%	***0.028***	***2.232***	1.088–4.576
housed on straw	92.9%	***0.032***	***0.625***	0.407–0.960
housed on sawdust	42.5%	0.890	1.019	0.780–1.330
housed on sand	01.4%	0.720	1.173	0.491–2.806
housing with solid manure	87.9%	0.217	1.325	0.848–2.070
housing with slurry channel	32.7%	***0.028***	***1.411***	1.039–1.918
availability of running yard	32.0%	0.773	1.040	0.796–1.360

F=frequency, p = significance, OR=Odds ratio
significance level ($p < 0.05$)

Table 2
Impact of housing of young cattle on being assigned MAP-positive herd status by using multivariable analysis.

Housing of young cattle	F	p	OR	Confidence interval
tethered stall	77.2%	0.472	0.800	0.435–1.470
open cowsheds	26.4%	0.653	1.164	0.600–2.258
Cubicles	13.8%	0.752	0.913	0.518–1.607
housing in deep litter systems	09.2%	0.668	1.130	0.645–1.981
housed on straw	90.9%	0.086	0.671	0.425–1.058
housed on sawdust	40.3%	0.850	1.027	0.777–1.357
housed on sand	00.4%	0.998	- *	- *
housing with solid manure	87.5%	***0.041***	***1.744***	1.023–2.974
housing with slurry channel	29.7%	0.697	1.160	0.660–1.321
availability of running yard	30.2%	0.432	1.224	0.847–1.473

* not calculable, F=frequency, p = significance, OR=Odds ratio
significance level ($p < 0.05$)

Table 3
Impact of livestock rearing on being assigned MAP-positive herd status using multivariable analysis.

Livestock rearing	F	p	OR	Confidence interval
separation of the calf from the mother immediately after birth	80.9%	0.440	0.884	0.646–1.209
colostrum from own mother only	97.4%	0.654	1.498	0.256–8.779
mixed colostrum from different cows	01.1%	0.634	0.547	0.046–6.546
rearing calves with whole milk	94.4%	0.743	1.140	0.522–2.488
rearing calves with sour milk	02.8%	0.433	0.694	0.278–1.730
rearing calves with milk replacer	05.0%	***0.047***	***0.472***	0.225–0.990
feeding waste milk to calves	38.2%	0.250	0.872	0.691–1.101
calf igloo housing	11.0%	0.079	1.345	0.967–1.872
individual pens for calves	79.5%	0.338	1.160	0.856–1.572

F=frequency, p = significance, OR=Odds ratio
significance level ($p < 0.05$)

2.11. Statistical analysis

The data from 2013 were descriptively described and analyzed using the IBM SPSS v27 (IBM Corp., Amrock, N.Y., USA) software. For statistical analysis a multiple logistic regression analysis was performed, with the herd status MAP positive or negative as dependent variable and the management factors as independent variables. Management factors were thereby combined into the statistical models according to the respective section of the questionnaire. Furthermore, a final model, including the significant factors from each of the individual analysis was calculated. This approach to analysis was chosen because the interest

Table 4
Impact of management at the farm on being assigned MAP-positive herd status by using multivariable analysis.

Management at the farm	F	p	OR	Confidence interval
presence of breeding bull	10.9%	0.736	1.068	0.728–1.568
breeding association	79.1%	0.092	1.313	0.956–1.803
additional purchase of cattle	51.2%	0.608	0.941	0.747–1.186
rearing offspring on farm	93.2%	0.713	1.112	0.631–1.959
external rearing of offspring	04.4%	0.777	0.904	0.451–1.814
availability of calving pen	17.5%	0.427	0.774	0.411–1.457
availability of sick cow pen	15.7%	0.324	1.392	0.721–2.686
common use of calving and sick cow pen	18.0%	0.378	0.814	0.515–1.287

F=frequency, p = significance, OR=Odds ratio
significance level ($p < 0.05$)

Table 5
Impact of management off the farm on being assigned MAP-positive herd status by using multivariable analysis.

Management off the farm	F	p	OR	Confidence interval
alpine pasturing	94.3%	0.952	1.018	0.567–1.829
sharing alpine pasture	82.7%	0.419	1.143	0.826–1.581
sharing pasture	23.9%	0.564	1.080	0.831–1.405
own pasture use	83.2%	0.704	1.060	0.784–1.434
sharing equipment	52.8%	0.733	0.963	0.773–1.198

F=frequency, p = significance, OR=Odds ratio
significance level ($p < 0.05$)

was in determining the effect of factors within an individual topic on the herd MAP status after controlling for the effect of other variables in the same topic. The variables of each model were checked for multicollinearity, using the phi- and Spearman-coefficients, both indicating no evidence for multicollinearity. For analysis of the structural data a Fisher's exact test was applied. Herd size data were transformed for statistical analysis using log10 transformation. The level of significance was $p < 0.05$ for all tests.

3. Results

Results of the farm survey from 2013 were compared with the MAP status of tested farms. Overall, 4718 surveys were included in the study. Of these farms 390 (8.3%) were assigned MAP-positive and 4328 (91.7%) MAP-negative status. The 50 questions were analyzed to identify factors that contribute to higher or lower chances of specific farms being MAP-positive.

Statistical analyses showed that four topics were significantly associated with MAP herd levels in the category "*housing of cattle*" and one in the category "*livestock rearing*".

3.1. Structural data

Questions about the structural data aimed to give an overview about farm size and cattle breeds. Neither farm size nor cattle breed had statistically significant impacts on the MAP herd status in this study. Although not significant ($p = 0.425$) it can be seen that the larger the farm (cattle count) the higher the chance of it being MAP-positive (Fig. 2). As only very few farms keeping more than 50 cattle were enrolled in the study, no further statistical analysis concerning the herd size was performed. The predominant breeds in participating farms were Austrian Simmental (51.1%), Brown Swiss (30.6%) and Tyrolean Grauvieh (16.6%) and there was no statistically significant results with the MAP-herd status ($p = 0.895$).

3.2. Housing of cattle

Over 90% of the farms enrolled used straw as bedding material, leading to nearly 88% of solid manure management. About 30% of the farms used housings with slurry channel systems. Additionally, 12% used both, solid manure management and slurry channel systems. In around 80% of farms, cattle were housed in tethered stall systems and 20% of farms used open cowsheds for their animals. About 4% of the farms employed deep litter housing for their cows and 9% for their young cattle up to six months, respectively. Sawdust was used as bedding material in more than 40% of farms (Tables 1 and 2).

Statistical analysis revealed that housing of cows with deep litter

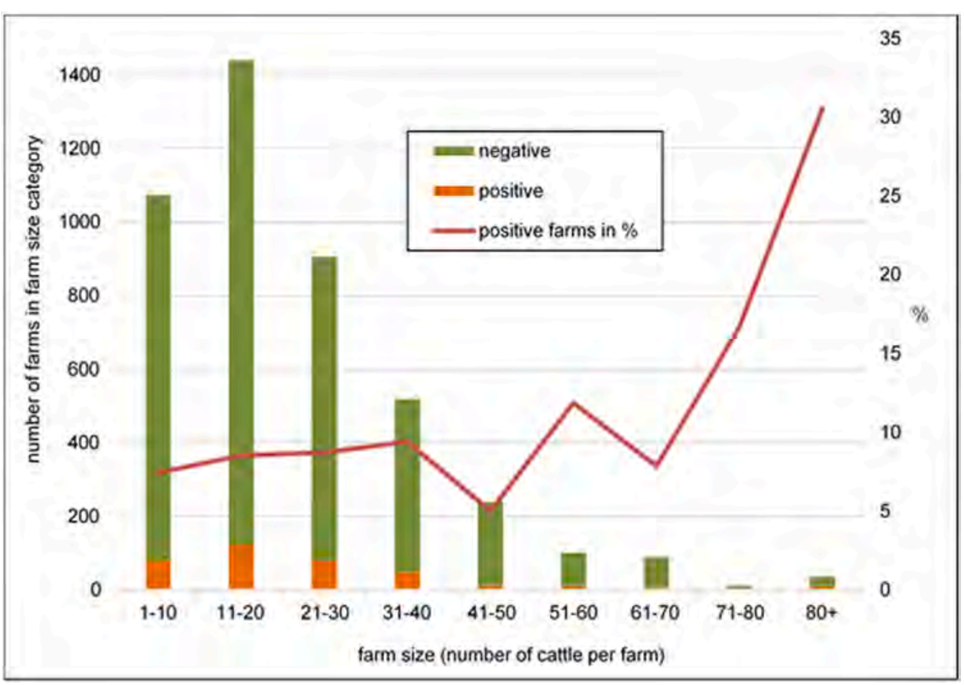

Fig. 2. Farms divided in decadic groups and their MAP-positivity rate with trendline.

housing systems and slurry channel systems significantly increased the OR of being MAP-positive in participating farms, while using straw as bedding material significantly decreased the OR (Table 1).

The use of solid manure in young cattle significantly increased the OR of being MAP-positive in the present study (Table 2).

3.3. Livestock rearing

Almost all participating farms fed their calves with colostrum from their mother (97%) and over 80% of farms separated the calf immediately after birth. Further, over 94% of farms used whole milk for calf rearing. Only 5% used milk replacer to rear their calves, whereas feeding waste milk was a common practice in about 38% of farms (Table 3).

Feeding calves with milk replacer significantly decreased the OR for a farm being MAP-positive. All other factors showed no significant association with the MAP herd status (Table 3).

3.4. Management practices

Nearly 80% of the farms belonged to a cattle breeding association and over 90% reared offspring on their own farm (Table 4).

Almost all participating farms used alpine pasturing (94%) on shared alps (83%). More than half (53%) of the farms shared equipment among themselves (Table 5).

None of these management practices showed a significant association with the MAP herd status (Table 5).

3.5. Final Model

When the significant variables from the statistical models 1–5 were used in a final model, only housing of cows with a slurry channel and rearing of calves with milk replacer remained significant (Table 6).

In Fig. 3 the OR for a MAP-positive herd status are shown for all 50 management factors evaluated in course of the present study.

4. Discussion

In the course of this study we focused on relationships between data collected with a questionnaire in 2013 and the overall MAP herd status (MAP-positive or -negative) from 2013 to 2018. If any positive animal was identified during the study period, it was assumed, that the infection had already been present in the herd for some time, although the actual timepoint of MAP introduction remains unknown.

Boot swap sampling for the detection of the MAP-herd status has a documented sensitivity of up to 90.6% when herds were sampled repeatedly (Eisenberg et al., 2013). Other studies calculated a sensitivity of 43.5% when two boot swabs were collected at one point of time (Wolf et al., 2016). However, since 66% of all farms were sampled at least twice, the sensitivity should be sufficiently high in the present study, as repeated sampling increases the sensitivity.

From a total of 5592 farms sampled, 7.81% were MAP-positive at least once. In addition, the comparison of the first investigation in 2013/2014 (8% MAP-positive) to the second in 2016/2017 (1% MAP-positive) indicates that the monitoring of MAP by the Tyrolean Animal Health Service could be a good working tool to control MAP in small scaled alpine agricultural systems. To elucidate possible risk factors for cattle farms, MAP-positive associations for each management factor were evaluated by a multivariable analysis, including calculation of OR.

Housing for cows with slurry channel systems was statistically significantly correlated with an increased chance for a herd of being MAP-positive in our study, both in the initial ($p = 0.028$, OR=1.411) and the final statistical model ($p = 0.003$, OR=1.438. This appears to be detrimental, contrary to the literature in this study.

Slurry channel systems are often used in combination with tethered housings, where a channel for slurry is placed behind the cows. Slurry channel systems can be a clean solution for cattle keeping, as feces can flow away from them under the surface and minimize contact with other animals and thereby reduce the transmission of pathogens. The higher chance of being MAP-positive in this case therefore disputes existing literature (Sweeney, 1994). Nevertheless, this finding could be indicative of malfunctioning channels and/or a generalized unsanitary environment. Poor ventilation can lead to increased aerosol formation, which can also affect transmission through droplet dispersion. Further, it is not always easy and, in some cases, not even possible to clean these channels adequately, especially in old premises.

Use of deep litter ($p = 0.028$, OR=2.232) in housing for cows was statistically significantly correlated with an increased chance for a herd of being MAP-positive in our study as well, although no significance could be found in the final model combining significant variables only ($p = 0.310$, OR=1.347).

In deep litter housing systems animals are kept on straw, which serves as long-term bedding, which is only cleaned out a few times each year. This provides a soft stall and good insulation if regularly littered. On the other hand it can serve as a reservoir for MAP and other pathogens (van Gastelen et al., 2011), which is in accordance with the results of the present study.

In contrast to the results on deep litter, the statistical analysis also showed that the use of straw ($p = 0.032$, OR=0.625) for housing cows significantly reduced the chance of being MAP positive. Again, this variable was not significant in the final model ($p = 0.215$, OR=0.775). Straw is predominantly used as a soft carpet pad and as a liquid binder in cattle housing. Due to its ability to bind liquids, the stalls are potentially drier than those without straw bedding. Dry environments provide poor reservoir conditions for MAP (Grewal et al., 2006). However, straw also is the basis for a deep litter barn (see above) or the creation of solid manure (see below). Therefore, the use of straw is difficult to consider individually.

Looking at the results of housing of young cattle, there is a significantly increased chance for a farm to be MAP positive if it uses solid manure in the initial ($p = 0.041$, OR=1.744), but not in the final model ($p = 0.102$, OR=1.372).

Solid manure is mainly produced in older barns, as it is used there for padding and absorbent material. This manure is usually removed daily and stored outside the barn in the form of a manure pile. The result of this study can be explained by the formation of a reservoir of MAP in dung heaps, to which the staff has daily contact. An old barn again is an indication of rather poorer ventilation and a structurally lower standard of hygiene. On the other hand, Grewal et al. (2006) has shown that monitored composting (manure piles) is a good option for the treatment of manure containing pathogens such as MAP. However, a minimum temperature must be reached over a certain period of time.

A clearly decreased chance of being MAP-positive was found in farms feeding calves milk replacer, which was statistically significant in our study, both in the initial ($p = 0.047$, OR=0.472) and the final model ($p = 0.010$, OR=0.339). Rearing calves with milk replacer is recognized

Table 6
Impact of the significant variables from Tables 1–5 on being assigned MAP-positive herd status using multivariable analysis.

Significant variables	F	p	OR	Confidence interval
housing cows in deep litter systems	3.6%	0.310	1.347	0.759–2.390
cows housed on straw	92.9%	0.215	0.775	0.517–1.160
housing cows with slurry channel	32.7%	***0.003***	***1.438***	1.127–1.836
housing of young cattle with solid manure	87.5%	0.102	1.372	0.939–2.005
rearing calves with milk replacer	5.0%	***0.010***	***0.339***	0.149–0.772

F=frequency, p = significance, OR=Odds ratio
significance level ($p < 0.05$)

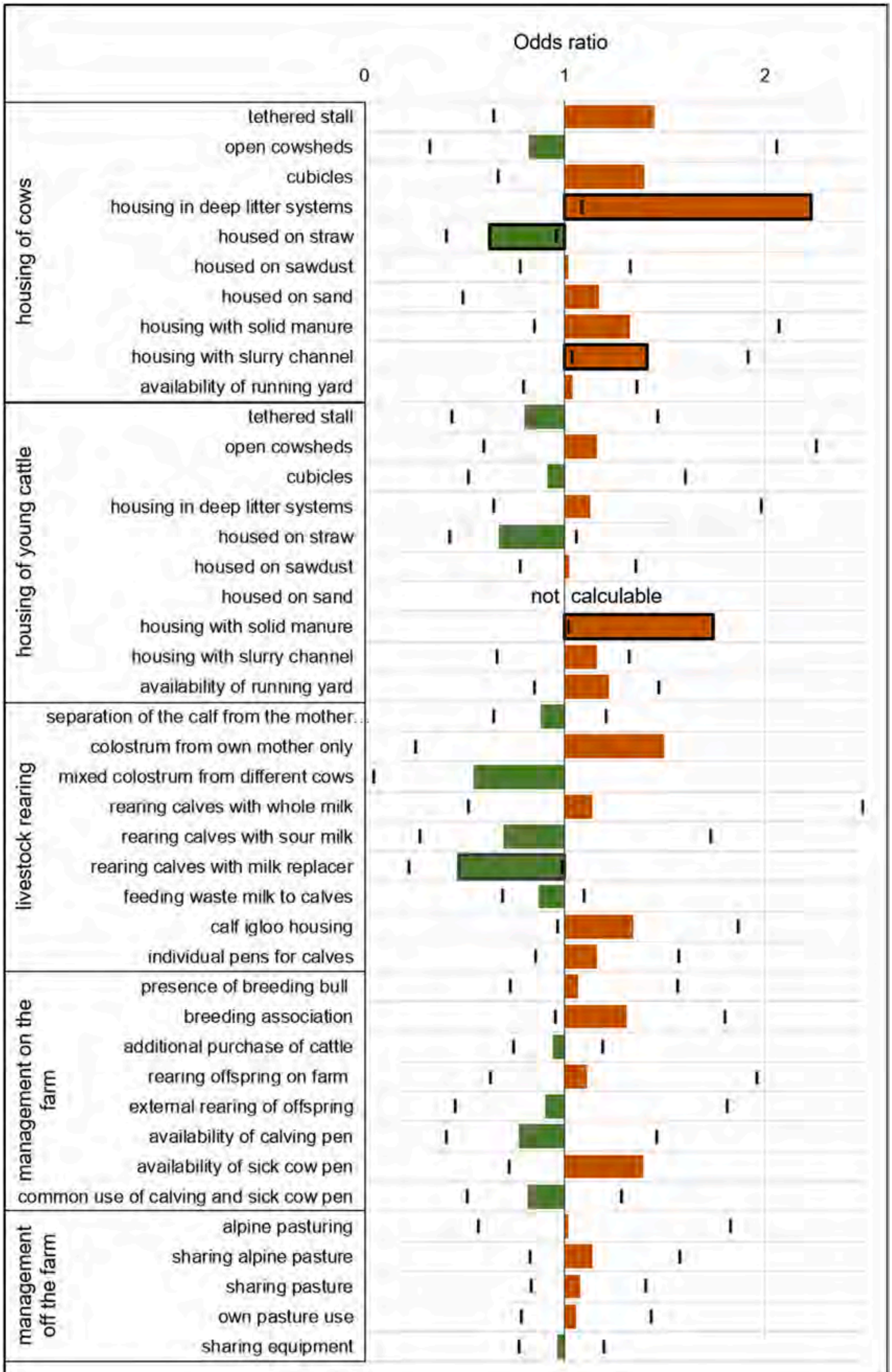

Fig. 3. Odds ratios of housing and management variables of participating farms (see supplements for details) for being assigned a MAP-positive herd status Strips indicate the 95%-confidence interval,boxed columns are significant($p < 0.05$).

to reduce the risk of MAP transmission (Grant et al., 2017). Milk replacers are based on milk that has been highly processed, including heating and drying. This near-sterile powder should be prepared with clean water in clean buckets before serving. If instructions are followed, this manner of feeding can effectively eliminate transmission of pathogens from mother to calf (Khol et al., 2017).

In this study, the relationship between farm size and MAP-positive-status was not significant ($p = 0.425$). High-turnovers, in terms of frequent purchases of cattle from unknown herds (Künzler et al., 2014) and frequent movements of workers among large farms, can be one general possibility for infection (Sayers et al., 2015). Alternatively, it has been proven that smaller barns can have an increased chance of being MAP-positive; as owners of smaller farms often work additionally on other facilities and relatively higher numbers of purchases are required for restocking than in larger farms (Puerto-Parada et al., 2018; Villamil et al., 2020). Due to the small sizes of the farms examined in this study, it is likely that many are operated part-time.

Surprisingly, our study did not indicate statistically significant associations favoring either increased or decreased chances of being MAP-positive for many management aspects previously related to the transmission of MAP. For example, in our study the sharing of pastures (lowland: $p = 0.564$, OR=1.080 / alpin: $p = 0.419$, OR=1.143) and solo alpine pasturing ($p = 0.952$, OR=1.018) were not correlate with an increased chance of a herd being MAP-positive. Animals from different herds sharing pastures can lead to disease spread. Horizontal transmission in this manner is a realistic risk for a pathogen such as MAP that is transmitted by the fecal-oral route (Slana et al., 2008). MAP also has a high tenacity in the environment (Rowe and Grant, 2006), which appears to be mediated via harboring of MAP in ubiquitous protozoa (Whan et al., 2006; Whittington et al., 2005). Further, cattle on alpine pastures come into contact with different wildlife species, which are also recognized as MAP reservoirs, for example, roe deer (Machackova et al., 2004), birds (Álvarez et al., 2006), mice, rats, hares, foxes (Florou et al., 2006) and various insects and bears (Kopenca et al., 2005). Therefore, it has long been assumed that sharing pastures was an important factor for MAP-transmission in Tyrol. Nonetheless, results of the present study indicate that this might be of minor importance. It is of course possible that this may be attributed to the fact that cattle are not introduced to alpine pastures, before they are several months old. In addition, stocking densities on these pastures is relatively low, and thus both factors may contribute to a decreased risk of MAP-transmission. The fact, that comparable few farms enrolled in the study were not sharing pastures, resulting in a small sample size in the unexposed group, may also have added to this result.

Moreover, the finding that purchasing cattle ($p = 0.608$, OR=0.941) and sharing equipment with other farms ($p = 0.733$, OR=0.963) were not related to a higher risk for being MAP-positive in the present study, disputes the literature regarding MAP transmission in cattle.

The purchase of subclinically-infected animals probably is the most common way to introduce MAP into a herd. Due to its pathogenesis and the chronic course of paratuberculosis, no clinical signs are present at the time of purchase and infected animals are generally purchased unwittingly (Valentin-Weigand, 2002; Klee, 2006; Künzler et al., 2014).

The sharing of equipment among farms is also recognized as an infection risk if it is not properly cleaned. And because of its high tenacity in the environment and feces, MAP can be transported over long distances in this manner (Santos et al., 2015).

The reason, why these two known risk factors for the transmission of MAP infections were not confirmed in our study remains unclear. However, they may be at least partially a result of the comparably low numbers of MAP-positive farms enrolled in the study, leading to a possible underestimation of some management factors. Additionally, both the number of shedders and the number of animals at risk are lower in small farms, which also may explain this result. Most scientific comparative studies conducted in larger agricultural structured areas, such as Spain, North America, Northern Germany and Denmark. The MAP-prevalence also is higher in these areas than in Tyrol.

More detailed investigations are necessary to investigate further into the transmission of MAP across small structured herds.

The results of the present study suggest that early separation of calves from their mothers decreases the chance of being MAP-positive but was not significant ($p = 0.440$, OR=0.884).

Feeding waste milk to calves also showed no significant association with the MAP herd status ($p = 0.250$, OR=0.872). This is in contrast to previous studies where feeding waste milk has been shown to increase the chance of MAP infection (Aust et al., 2013). Previous studies have also shown that MAP can be transmitted to calves via milk, thereby contributing to transmission of the disease within a farm (Slana et al., 2008). The reason why the feeding of waste milk to calves seemed not to contribute to a positive MAP-herd status remains unclear and needs further investigation.

The same is true for feeding colostrum to calves from their mother only, which was not correlated with the MAP-herd status ($p = 0.654$, OR=1.498), although previously reported to reduce MAP-transmission (Nielsen et al., 2008). As 97% of farms participating in our study fed calves with colostrum solely from their mother and only 1% used mixed colostrum respectively, interpretation of this result is quite limited.

To summarize, results of the present study revealed five statistically significant factors that might influence the OR of a farm being MAP-positive or -negative within participating herds. Farms rearing calves with milk replacer had a decreased OR of being MAP-positive. Alternatively, housing cattle on deep litter and/or using sawdust as bedding material increased the OR for a MAP-positive herd status. The same was found for premises with slurry channel systems. However, only the variables “rearing calves with milk replacer” and “housing cows with a slurry channel system” remained significant in the final statistical model.

Furthermore, farm size did not influence the chance of being MAP-positive, which is in contrast to most literature.

4.1. Strength and weaknesses of the study

This study covers the status of 5592 farms over a period of 5 years. This large data set is one of the strengths of this work and increases the power of the results. The extensive questionnaire additionally provides a detailed and realistic reflection of farm processes and farm structures.

On the other hand, a weakness of this study is that not all farms were sampled with an equal frequency. From the data it is furthermore not possible to determine when and by which events MAP was introduced into a farm. It is also not possible to determine which of the applied measures in the individual positive farms led to a consecutive negative result. Also, different types of housing and operating conditions frequently overlap on farms enrolled, so that the validity of the result of an isolated question must in any case be seen in context. For this reason, a final questionnaire in 2018 to review operating conditions would have been desirable.

5. Conclusions

Surprisingly, procedures commonly believed to increase the spread of pathogens, including MAP, such as sharing lowland or alpine pastures as well as farm equipment, seemed to have no influence on the MAP herd status of participating farms. Nevertheless, some known association of management and housing factors with the herd MAP-status (use of milk replacer and manure management) were confirmed in the present study. Contradictions between literature and the results of this study might depend on the small family-based agricultural structure in the Tyrolean province investigated and its traditional characteristics.

The results of the present study contribute to the understanding of the spread of MAP in traditional cattle faming. Nevertheless, more studies are required to further elucidate the dynamics of JD in alpine farms.

Declaration of Competing Interest

The Authors declare that there is no conflict of interest.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.prevetmed.2023.105999.

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