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**COMPREHENSIVE QUANTITATIVE RISK ASSESSMENT ON THE
PRESENCE OF *Mycobacterium avium* subsp. *paratuberculosis*
IN MILK AND MILK PRODUCTS**

Doctoral dissertation

**CELOVITA KVANTITATIVNA OCENA TVEGANJA ZA
PRISOTNOST *Mycobacterium avium* subsp. *paratuberculosis*
V MLEKU IN MLEČNIH IZDELKIH**

Doktorska disertacija

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Comprehensive quantitative risk assessment on the presence of *Mycobacterium avium* subsp. *paratuberculosis* in milk and milk products

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ABSTRACT

Key words: Cattle diseases – epidemiology; risk assessment – methods; paratuberculosis – transmission – epidemiology; prevalence; *Mycobacterium avium* subsp. *paratuberculosis*; milk – microbiology; food safety; biological models

The objective of this thesis was to assess the risk of exposure to *Mycobacterium avium* subsp. *paratuberculosis* (MAP) via milk and milk products for the Slovenian consumer. MAP is suspected to be linked to Crohn's disease and some other chronic human diseases. Although causality has not yet been confirmed, it is important to improve the understanding of the risk of human exposure. Cattle are believed to be the main source of MAP for humans. In cattle, MAP causes paratuberculosis or Johne's disease, a worldwide endemic disease with large economic consequences. Due to the lack of empirical data, we applied two models as tools for obtaining data on the epidemiological situation of MAP, specific for the Slovenian dairy sector. A stochastic compartmental model was developed to study the within-herd spread of MAP in a typical Slovenian dairy herd and a susceptible-infectious model on the temporal network of cattle movements to estimate the prevalence of MAP using four different scenarios for probabilities of transmission. The obtained results were used in the quantitative risk assessment model with Monte Carlo simulations to assess possible human exposure to MAP via milk and milk products. Additionally, network analysis of Slovenian cattle movements was performed to estimate the prospect of targeted MAP intervention measures. The results suggest that network analysis may provide support in the optimization of paratuberculosis control in Slovenia. The compartmental model showed a similar within-herd prevalence of MAP in Slovenia as was previously estimated, which was opposite to what we hypothesized. Concerning the underlying assumptions and uncertainties, we estimated that the overall risk of exposure to MAP via milk and milk products for the Slovenian consumer is low. The quantitative exposure assessment was performed for raw milk, pasteurised whole milk and ultra-high temperature treated milk; nevertheless, we believe that the results can be extended, at least in part, to other milk products at the respective level of production. For people consuming raw milk and raw milk products from farms with paratuberculosis, the risk of exposure to MAP is high. However, the simulated amount of MAP per litre of raw bulk tank milk was low. It is important to emphasise that the risk of exposure to MAP via pasteurised retail milk and via other processed milk is most likely insignificant. Our results could provide support for informed decision-making in MAP intervention measures in Slovenia and in case the link between MAP and human diseases is confirmed also as a start for possible risk mitigation efforts. These results could be applicable to other countries with a similar dairy farm structure.

IZVLEČEK

Ključne besede: Bolezni goveda – epidemiologija; ocena tveganja – metode; paratuberkuloza – prenos – epidemiologija; prevalenca; *Mycobacterium avium* subsp. *paratuberculosis*; mleko – mikrobiologija; varnost živil; biološki modeli

Cilj disertacije je bil oceniti tveganje za izpostavljenost bakteriji *Mycobacterium avium* subsp. *paratuberculosis* (MAP) preko mleka in mlečnih izdelkov za slovenskega potrošnika. Domneva se, da je MAP lahko povezana s Crohnovo boleznijo in nekaterimi drugimi kroničnimi boleznimi ljudi. Čeprav vzročnost še ni bila dokazana, je pomembno izboljšati razumevanje tveganja za izpostavljenost. Glavni vir MAP za ljudi je najverjetneje govedo. MAP pri govedu povzroča paratuberkulozo ali Johne-jevo bolezen, ki zmanjšuje ekonomsko učinkovitost govedoreje po vsem svetu. Zaradi pomanjkanja empiričnih podatkov smo razvili dva modela za pridobitev podatkov o možnem epidemiološkem stanju v sektorju mleka v Sloveniji. S stohastičnim razrednim modelom smo preučili širjenje MAP v tipični slovenski čredi krav molznic. Z modeliranjem širjenja MAP na časovnem omrežju s tako imenovanim SI modelom (angl. *susceptible-infectious*; dovzet-enficiran) in štirih scenarijev verjetnosti prenosa MAP pa smo ocenili možno prevalenco. Pridobljene rezultate smo uporabili v modelu kvantitativne ocene tveganja s simulacijami Monte Carlo za oceno možne izpostavljenosti človeka bakteriji MAP preko mleka in mlečnih izdelkov. Poleg tega smo izvedli analizo omrežja premikov goveda v Sloveniji, da bi ocenili možnost izvajanja ciljanih intervencijskih ukrepov. Rezultati kažejo, da analiza omrežja lahko nudi podporo pri optimizaciji nadzora paratuberkuloze v Sloveniji. V nasprotju z našo hipotezo je razredni model pokazal podobno razširjenost MAP znotraj črede, kot so ocenile predhodne raziskave v Sloveniji. Z upoštevanjem danih predpostavk in negotovosti smo ocenili, da je splošno tveganje za izpostavljenost MAP preko mleka in mlečnih izdelkov za slovenskega potrošnika majhno. Kvantitativno oceno izpostavljenosti smo izvedli za surovo mleko, pasterizirano polnomastno mleko in mleko, obdelano pri ultra visoki temperaturi, vendar menimo, da se lahko rezultati do neke mere prenesejo tudi na druge mlečne izdelke na zadevni ravni proizvodnje. Za ljudi, ki uživajo surovo mleko in surove mlečne izdelke s kmetij s paratuberkulozo, je tveganje za izpostavljenost MAP visoko. Vendar je bila simulirana količina MAP na liter surovega mleka v rezervoarju za mleko na kmetiji nizka. Pomembno je poudariti, da je tveganje za izpostavljenost MAP preko pasteriziranega mleka v maloprodaji in preko drugega predelanega mleka najverjetneje zanemarljivo. Naši rezultati bi lahko nudili podporo za informirano odločanje o intervencijskih ukrepih za nadzor MAP v Sloveniji. V primeru, da bo povezava med MAP in boleznimi pri človeku potrjena, lahko naša raziskava služi kot dober začetek prizadevanj za zmanjšanje tveganja. Dobljeni rezultati so lahko uporabni tudi za druge države, ki imajo podobno strukturo mlečnih gospodarstev.

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LIST OF ABBREVIATIONS AND SYMBOLS

AIC	Akaike Information Criterion
B	Compartment buying
C	Compartment culled
CC	Collection centres
CFU	Colony forming unit
CI	Confidence interval
E_h	Latent cows on high shedding path
E_l	Latent cows on low shedding path
EFSA	European Food Safety Authority
ELISA	Enzyme-linked immunosorbent assay
EMULSION	Epidemiological Multi-Level Simulation Framework
EX	Exhibitions
E_y	Latent young animals
FA	Fairs
FAO	Food and Agriculture Organization of the United Nations
FH	Farm holdings
GIC	Giant in component
GOC	Giant out component
GSCC	Giant strongly connected component
GWCC	Giant weakly connected component
H	High shedding cows
HS	Herd status
HTST	High-temperature short time pasteurisation
I_h	Infected cows on high shedding path
I_l	Infected cows on low shedding path
KIS	Agricultural Institute of Slovenia
m	Milk per herd (litres/day)
MAP	<i>Mycobacterium avium</i> subsp. <i>paratuberculosis</i>
MAP_{ext}	External milk contamination (log CFU)
MAP_{fc}	MAP from faeces per clinical cow (log CFU/litre)
MAP_{fs}	MAP from faeces per subclinical cow (log CFU/litre)
MAP_{herd}	Bulk tank raw milk contamination on farm level (log CFU)
$MAP_{herd\ past}$	Pasteurised whole milk contamination on farm level (CFU/litre)
$MAP_{herd\ raw}$	Raw milk contamination on farm level (CFU/litre)
$MAP_{industry}$	Silo milk contamination on dairy industry level (log CFU)
$MAP_{industry\ past}$	Pasteurised whole milk contamination on dairy industry level (CFU/litre)
MAP_{int}	Internal milk contamination (log CFU)
MAP_{mc}	MAP shedding in milk per clinical cow (log CFU/litre)

MAP_{ms}	MAP shedding in milk per subclinical cow (log CFU/litre)
m_c	Milk per clinically infected cow (litres/day)
m_h	Milk production per healthy cow (litres/day)
MP	Mountain pastures
m_s	Milk per subclinically infected cow (litres/day)
N	Number of dairy cows per herd
N_c	Number of clinically infected cows
N_h	Number of healthy cows
N_i	Initial number of adult animals; dairy cows
N_s	Number of subclinically infected cows
OIE	World Organisation for Animal Health
p	Probability of transmission
P	Animal-level prevalence
PA	Pastures
P_c	Proportion of clinically infected cows
PC	Percentile
PCR	Polymerase chain reaction
P_s	Proportion of subclinically infected cows
S_1	Herd level prevalence, best-case scenario (%)
S_2	Herd level prevalence, middle scenario (%)
S_3	Herd level prevalence, estimated scenario (%)
S_a	Susceptible cows
SI model	Susceptible-infectious model
Spearman's ρ	Spearman's rank correlation coefficient
SURS	Statistical Office of the Republic of Slovenia
S_y	Susceptible young animals
UHT milk	Ultra-high temperature treated milk
UVHVVR	Administration of the RS for Food Safety, Veterinary Sector and Plant Protection
WHO	World Health Organization
Y	Number of young animals; calves and heifers
Y_i	Initial number of young animals; calves and heifers
α	Proportion of latently infected young animals entering the high shedding path
β	Infection probability given contact
β_e	Transmission rate for latently infected cow (/day)
β_h	Transmission rate for high shedding cow (/day)
β_{hh}	Transmission rate from latently infected heifer to susceptible heifer (/day)
β_i	Transmission rate for infected cow (/day)
β_{ye}	Transmission rate from latently infected cow to susceptible calf (/day)
β_{yh}	Transmission rate from high shedding cow to susceptible calf (/day)
β_{yi}	Transmission rate from infected cow to susceptible calf (/day)

β_{yy}	Transmission rate from latently infected calf to susceptible calf (/day)
γ_h	Proportion of latent calves per high shedding cows
γ_p	Proportion of latent calves per latently infected and infected cows
δ	Rate of buying in new animals (/day)
δ_p	Transition rate from buying to latently infected and infected cow compartments
δ_s	Transition rate from buying to susceptible cow compartment
ε	MAP reduction with pasteurisation (log CFU)
η_c	Proportion of milk produced per subclinical cow
η_s	Proportion of milk produced per subclinical cow
λ_a	Force of infection for adult animals (/day)
λ_y	Force of infection for young animals (/day)
μ_a	Culling rate for all cows except high shedding cows (/day)
μ_b	Birth rate for all cows except high shedding cows (/day)
μ_{be}	Birth rate of latently infected female calves and direct transmission from mothers
μ_{bh}	Birth rate for high shedding cows (/day)
μ_{bs}	Birth rate of susceptible female calves
μ_h	Culling rate for high shedding cows (/day)
μ_r	Culling rate or sale of redundant young animals (/day)
μ_y	Culling or sale of young animals (/day)
ρ_{eh}	Replacement of culled animals with own animals; from E_y to E_h
ρ_{el}	Replacement of culled animals with own animals; from E_y to E_l
ρ_s	Replacement of culled animals with own animals; from S_y to S_a
σ	Transition rate from latent to infected cow (/day)
τ	Contact probability
φ	Milk filter efficiency
ω	Progression from an infected cow on high shedding path to high shedding cow (/day)

1 INTRODUCTION

Mycobacterium avium subsp. *paratuberculosis* (MAP) may represent a risk for animal and human health. In cattle, MAP causes paratuberculosis or Johne's disease, which reduces the economic efficiency of the cattle industry worldwide. In Europe, the animal level prevalence in cattle is believed to be approximately 20%, with the lowest estimates of 3–5%. The highest prevalence is reported in dairy cattle. Paratuberculosis is also common in ruminants in Slovenia. In 2008, it was estimated that 18.49% of cattle herds were infected, while the estimated true-prevalence at the animal level was 3.96%. MAP is also able to infect and cause clinical signs in many other domestic and wild animal species, including non-human primates.

In humans, MAP is assumed to be an infectious agent of Crohn's disease and some other diseases. Although the discussions about whether or not MAP is a zoonosis are ongoing and controversial, the fact is that viable MAP is found in many potential sources for human exposure. Potential sources of human infection include contaminated food, water, environment and direct contact with infected animals. Some researchers believe that people consuming normal diets might be repeatedly exposed to MAP. Even though details about the nature and consequences of the infection are unknown, it is important to improve the understanding of the risk of human exposure.

An objective and transparent process of assessing the risk of an adverse event, in our case the risk of human exposure to MAP, is defined and integrated into the risk analysis framework. A structured and science-based approach to risk analysis related to food safety is proposed by different international organizations, e.g. the World Organisation for Animal Health (OIE), the European Commission, the European Food Safety Authority (EFSA) and the World Health Organization (WHO). We focused on quantitative risk assessment, which usually involves the development of different types of models. In mathematical modelling, the real system is simplified to such an extent that it can be systematically analysed and described by mathematical formulas. We used modelling to overcome the lack of empirical data and the problem of inadequate tests for diagnosing MAP infections since it allows the use of published information and expert opinions.

1.1 RESEARCH OBJECTIVES

Although various studies present MAP models, risk estimates cannot be simply transferred to another region or to a different time period. Differences in environmental factors, the population of susceptible animals, production systems, the legislation system of the country and consumer behaviours have to be considered. Our objective was to assess the risk of exposure to MAP via milk and milk products for the Slovenian consumer.

1.2 RESEARCH HYPOTHESES

The research aimed to test the following hypotheses:

1. The risk of human exposure to MAP by consumption of milk and milk products in Slovenia is not negligible.

2. The developed compartmental model will show higher prevalence of MAP in Slovenia compared to previous estimates.
3. The analysis of Slovenian cattle movements will show that, in order to reduce MAP spread and consequently reduce the human exposure via milk and milk products, it would be particularly useful to implement intervention measures on premises with higher centrality measures.

2 LITERATURE REVIEW

2.1 *Mycobacterium avium* subsp. *paratuberculosis*

Taxonomically MAP belongs to the family *Mycobacteriaceae*, genus *Mycobacterium*, more specifically to the *Mycobacterium avium* complex, which includes two species: *M. avium* and *M. intracellulare*. MAP is one of four subspecies of *M. avium*. MAP was first reported in Europe in 1895 when it was isolated from cattle with chronic diarrhoea and massive infiltration of the intestinal tract. Johne and Frothingham (1895) described it as a “peculiar case of tuberculosis in cattle” (McGovern, 2019).

Subtypes of MAP are divided into two main strain types named by the animal species from which they were primary isolated. Type I or S strain was originally isolated from sheep and is able to infect other small ruminants, cattle and deer. Type II or C strain was first isolated from cattle and is connected to infections with a broad range of ruminant and non-ruminant domestic and wildlife hosts. Transmission of C and S strains between sheep and cattle is possible but uncommon (CFSPH, 2007). In addition to genotypic and host differences, the two types of MAP differ in incubation period on a culture media. The C strain type needs to be incubated from four to 16 weeks, while the S strain type needs from four months to one year (Stevenson, 2010). An intermediate strain called type III strain, which is mostly isolated from domestic small ruminants, has also been identified (Stevenson et al., 2015).

MAP characteristics are described under the Results in chapter 4.1.1 MAP characterization.

2.1.1 Disease in animals

MAP is a multi-host pathogen, with the main reservoir and host probably being livestock ruminants. In cattle, MAP causes paratuberculosis, also known as Johne’s disease. Paratuberculosis is a worldwide endemic disease with large economic consequences (Barkema et al., 2010; Cho et al., 2013; Sweeney, 1996). The disease has a slow progression with a long incubation period and manifests as chronic and progressive gastroenteritis. The main clinical sign is diarrhoea, which is intermittent at the beginning but can progress to being persistent. Profuse diarrhoea leads to malabsorption, loss of plasma proteins, weight loss, and in some cases death (Sweeney, 1996; Collins, 1997).

MAP was found to be able to infect and cause clinical signs in more than 50 domestic and wild animal species, including non-human primates. On top of that, it was isolated from almost 40 additional animal species, which may act as vectors (EFSA, 2017). The transmission between wild and domestic animal species and sustainable MAP infections in wild animal populations indicate that a wildlife reservoir may play a role in disease persistence in regions where the disease is not endemic in the livestock population (Stevenson et al., 2009). Besides cattle, some of the susceptible animal species in our region are sheep, goat, chicken, dog, rabbit, fox, mouflon and deer. Based on the isolation of MAP from these species, the potential vectors in our region could be mouse, rat, wild boar and some species of birds (EFSA, 2017).

The disease is incurable (Sweeney, 1996; Collins, 1997); however, antimicrobial drugs can slow or prevent the progression of the infection. The treatment with antimicrobial drugs in livestock is not cost-effective and therefore not practised. Use of these drugs may be justified in high value animals

with valuable genetic characteristics, great sport or production performance or in pet animals (EFSA, 2017).

2.1.2 Zoonotic potential

There are reasonable grounds to suspect that MAP may present a risk for human health (Waddell et al., 2015). It is assumed to be connected with Crohn's disease, a chronic inflammatory bowel disease (Hermon-Taylor, 2001). There are limited data available on the prevalence and incidence of Crohn's disease in Slovenia, but they estimated that in 2016 there were approximately 2,000–2,500 patients (Zupančič et al., 2016). The study on the incidence rate of pediatric inflammatory bowel disease showed that the annual incidence of Crohn's disease per 100,000 children and adolescents in Slovenia increased from 3.6 in 2002 to 4.6 in 2010 (Urlep et al., 2015). A systematic review of the worldwide incidence and prevalence conducted by Molodecky et al. (2012) showed that Europe had the highest incidence of Crohn's disease with 12.7 new patients per 100,000 people per year. 75% of the reviewed articles reported a statistically significant increase in incidence over time. There are no estimates of the financial disease burden for Slovenia; however, the estimates for other countries showed that it is significant. The direct treatment costs are minor (approximately EUR 20,000 per patient) compared to the indirect disease costs as a result of reduced productivity, extra sick leave, lost wages due to premature retirement (approximately EUR 675,000 per patient), lost work hours for caregivers and premature death (Rao et al., 2017; Kuenzig et al., 2019). MAP was also linked to some other chronic human diseases (Waddell et al., 2016a), but there is still no consensus on whether or not MAP is a zoonosis (Kuenstner et al., 2017; Robertson et al., 2017). Nevertheless, it is important to improve the understanding of the risk of human exposure.

Many potential sources of human exposure to MAP were identified, namely contaminated food (e.g. pasteurised and unpasteurised milk, cheese and other dairy products, raw meat), water, environment and direct contact with infected animals. Waddell et al. (2016b) stated that people consuming normal diets might be repeatedly exposed to MAP. Even though possible exposure was established, there are still a lot of uncertainties and unknowns linked to MAP as a risk for human health. For example, the relative importance of each route of exposure, minimum infective dose and exposure time are unknown.

Cattle are believed to be the main origin of MAP for human exposure (Mihajlovic et al., 2011) and the most studied sources of exposure are milk and milk products. Garcia et al. (2015) pointed out that in the case of a confirmed link between MAP and Crohn's disease in humans, we can expect significant economic losses due to public health scare (Groenendaal and Zagmutt, 2008). The losses would disproportionately affect the dairy industry making the paratuberculosis control programmes economically beneficial and farmers more willing to participate (Garcia et al., 2015). This is especially important for the dairy sector in Slovenia since the milk output accounts for almost a third of the whole livestock output (Bedrač et al., 2018).

More on human exposure and potential consequences is described under the Results in chapter 4.1.2 Potential risk.

2.2 PARATUBERCULOSIS IN CATTLE

Paratuberculosis usually occurs as a subclinical infection, but some animals develop a chronic wasting disorder, which results in weight loss, decreased milk production and pregnancy rate and increased culling and mortality rate (Smith et al., 2010; Logar, 2012). The cost of this disease and its control in the cattle industry was shown to be high (Cho et al., 2013). Even though subclinically infected animals appear healthy, they are associated with financial losses (McAloon et al., 2015).

2.2.1 Transmission

The faecal-oral transmission is believed to be the most common route of within-herd transmission of MAP (Sweeney, 1996). Infection may also be spread with colostrum and milk from infected animals and intrauterine transmission. However, the main risk of infection for calves is posed by faecal contamination of the udder (Collins et al., 1994). Calves can also acquire the infection from other calves (van Roermund et al., 2007). Additionally, an alternative route of transmission with bioaerosols has been suggested (Eisenberg et al., 2012). It is assumed that the highest risk for MAP spread among cattle is posed by cattle and not by other reared animal species (ACMSF, 2016).

The main route of paratuberculosis spread between cattle herds is believed to be by movements of infected animals (Fecteau and Whitlock, 2010). Subclinically infected animals are considered the most important factor contributing to the spread of the disease into naive herds (Fecteau, 2018). Marquetoux et al. (2016a) coupled network analysis of livestock movements and genotyping of MAP strains on farms and showed that between-farm transmission of MAP is significantly associated with movements of animals from one farm to another. The same result was obtained by connecting the network analysis of livestock movements with the disease statuses of farms (Rossi et al., 2017a).

2.2.2 Within-herd dynamics

Calves are the most susceptible and with aging of the animals the susceptibility decreases. Nevertheless, infections of adult animals occur more often and may be more important in the on-farm persistence of the disease than was previously expected (Espejo et al., 2013; Schukken et al., 2015, Nigsch et al., 2019). The progression of the infection follows two different paths. Some animals become constant high-shedders and manifest clinical signs months or years after the infection, while other animals shed MAP in various amounts intermittently or not at all and remain asymptomatic (Mitchell et al., 2015a). Another reason that infected animals may never show clinical signs is that their productive life can be shorter than the progression of the disease. It was estimated that only 7% of infected animals progress to the clinically infected stage (Mitchell et al., 2015b). Whitlock and Buergelt (1996) described the proportion of animals that show clinical signs of the disease and those that can be detected as “the tip of the iceberg”. Based on the disease progression, MAP detectability and testing objectives, different classification systems for health states of animals in herds with paratuberculosis were suggested. Most of them describe three to four stages (Whitlock and Buergelt, 1996; Nielsen and Toft, 2008; Nielsen et al., 2009), however the within-herd dynamics of the disease was shown to be more complex. Smith et al. (2015) developed a compartmental model with five MAP positive health states for adult animals and demonstrated that adult infections and two paths of progression may play an important role in disease dynamics on a dairy farm.

2.2.3 Diagnostic tests

Different diagnostic tests for detecting MAP cells or an immune response to MAP infection are available. Although the specificity of most tests is high (usually close to 100%), the sensitivity is poor (often below 70%), especially in the early stage of infection. Tests for direct detection of MAP cells are smears with Ziehl-Nielsen staining, conventional, radiometric or tissue culture methods and molecular methods (EFSA, 2017). The latter are different types of polymerase chain reaction (PCR), namely conventional gel-based PCR, reverse transcriptase PCR, nested PCR and quantitative real-time PCR. PCR methods have the highest sensitivity but the majority of them are not able to distinguish between viable and nonviable cells which is of major importance when assessing the risk of human exposure (Robertson et al., 2017).

Tests for detection of the organism's immune response to MAP infection are enzyme-linked immunosorbent assay (ELISA) for detecting humoral immune response and detection of cell-mediated immunity with delayed type hypersensitivity and increased cytokine production (EFSA, 2017). Besides the low sensitivity of these tests, the long incubation period hinders a correct diagnosis of infection. MAP can be shed in faeces before the immune response yields positive test results (Van Schaik et al., 2003a; Van Schaik et al., 2003b; Beaver et al., 2017), however the shedding of MAP in faeces can be intermittent (Fecteau, 2018). In addition, the cost of sensitive diagnostic tests is an issue when designing feasible and effective control programmes.

2.2.4 Control programmes

Whittington et al. (2019) reviewed the control of paratuberculosis in 48 countries. They found that only 22 countries had formal control programmes. The main reasons for control were animal health considerations followed by the protection of market access and public health concerns. Most commonly used approaches for control were culling of clinically infected animals, improved hygiene in calf nurturing, prevention of disease introduction into a herd, management of the environment and pastures, communication and herd certification programmes. Vaccination was used only in seven countries. None of the control measures is effective on its own; therefore, control strategies usually consist of different combinations of practices and tools.

For example, some of the issues with vaccination are cross-reaction with bovine tuberculosis, differentiation between infected and vaccinated animals and although animals may not show clinical signs, they can still shed MAP. More on MAP resistance and survival outside the host is described under the Results in chapter 4.1.1 MAP characterization. Based on many knowledge gaps some researchers pose the question if eradication of MAP is possible (Barkema et al., 2018). Nevertheless, only Norway and Sweden stated that the objective of the control programme was the eradication of paratuberculosis, while for other countries the major objective was the reduction in prevalence (Whittington et al., 2019).

In Slovenia, there is currently no formal paratuberculosis control programme. However, some cattle herds are participating in the on-going study on paratuberculosis prevalence and herd level risk factors. One of the aims of this study is to design and implement herd specific control measures.

Since the disease is listed by the OIE, it is mandatory to notify the detection of paratuberculosis in cattle, sheep and goats in Slovenia. The Administration of the Republic of Slovenia for Food Safety, Veterinary Sector and Plant Protection (UVHVVR) has to submit bi-annually and yearly reports to the OIE (Rules on animal diseases, 2007).

2.2.5 Prevalence

The estimates of within-herd prevalence vary considerably (Nielsen and Toft, 2009), but even if the mortality in a herd stays low for years, half of the animals may be subclinically infected (Collins, 2016). In Europe, the animal level prevalence in cattle is believed to be approximately 20%, with the lowest estimates of 3–5% (Nielsen and Toft, 2009). The highest prevalence is reported in dairy cattle, where for many major dairy-producing countries estimates of infected herds range from 20% to 80% per country (Collins, 2016). A more recent study by Whittington et al. (2019) reported that from 48 countries included in the survey approximately half had more than 20% of herds infected. In some developed countries (e.g. Italy, France, Germany, the United Kingdom) prevalence exceeded 40%. Even though the reported prevalences were high, the authors warned that underreporting and underestimating paratuberculosis prevalence are both common. Additionally, comparison of prevalences between countries is difficult, because of the use of different definitions for prevalence, various diagnostic tests, sometimes with unknown sensitivity, and selection of different samples and sampled animals (Nielsen and Toft, 2009; Whittington et al., 2019).

Paratuberculosis is also common in ruminants in Slovenia (Ocepek et al., 1999). The last herd level prevalence study in Slovenia was conducted in 2008. In 20% of cattle herds from all parts of Slovenia, they sampled animals older than two years and used two types of ELISA tests for determining the presence of antibodies. For screening, they used a lower-cost in-house ELISA test and confirmed the positive results with a commercial ELISA kit with established specificity and sensitivity. They estimated that 18.49% of cattle herds were infected, while the estimated seroprevalence of paratuberculosis at the animal level was 3.96% (Kušar et al., 2011). Ocepek et al. (2002) argued that the low disease prevalence in Slovenia could be partly explained with the existence of numerous small herds since the spread of MAP is more probable within a herd than between different herds.

Logar et al. (2012) conducted a study where they compared four combinations of methods and samples for detection of MAP in subclinically infected dairy cattle. They sampled faeces and milk from animals in different age groups in herds with a history of paratuberculosis. They used real-time PCR after high-efficiency DNA extraction from faeces, faecal culture, milk real-time PCR and milk ELISA. The proportion of MAP positive samples were 89%, 19%, 36% and 1%, respectively. They stated that their culture results suggest the apparent prevalence of equal to or more than 20%.

2.3 RISK ANALYSIS

Food-borne zoonoses are of particular relevance to public health, as they may pose a health risk for consumers. Furthermore, animal diseases have an impact on revenue, markets and trade; thus, they represent a potential risk of large losses for the economy and, indirectly, also for consumers and taxpayers (Dijkhuizen and Morris, 1997; OECD, 2013; Shaw, 2019). Risk analysis aims to provide an objective and transparent process of assessing the risks of adverse events to enable informed and

defensible decision-making (OIE, 2016). A structured and science-based approach to risk analysis regarding animal health and food safety is proposed by different international organizations: e.g. OIE (Murray, 2004a; Murray, 2004b; OIE, 2016), the European Commission (European Commission, 2015), EFSA (EFSA, 2012), WHO and the Food and Agriculture Organization of the United Nations (FAO) (WHO/FAO, 2009).

The main reason for the differences between frameworks lies in their objectives. For example, the OIE standards and recommendations are related to managing risks associated with the importation of animals and animal products and assessing the likelihood of entry as well as biological and economic consequences. However, the methodology is applicable whenever the goal is to manage risks of introduction or spread of infectious diseases (Murray, 2004a; Murray, 2004b; OIE, 2016). The objective of the WHO/FAO framework is to provide support for better understanding and management of microbiological food safety risks. The result of this framework is the incidence of effects on human health that can be attributed to e.g. certain food, pathogen or process (WHO/FAO, 2009). Components and steps of these two frameworks are shown in Figure 1.

While there are some differences in approaches among the aforementioned frameworks, in general, risk analysis consists of four components. The first component is *hazard identification*. After the pathogenic agent of interest has been defined, qualitative or quantitative *risk assessment* takes place. The outputs of this step serve as a basis for *risk management* since every proposed measure for risk reduction needs to be compared to the status quo (OIE, 2016). Throughout the risk analysis process, effective *risk communication* with all stakeholders is important as it facilitates the acceptance of proposed preventive or control measures. Risk analysis can be conducted at different epidemiological units; at the animal, herd or specific regional level. It also applies to international disease management, as well as for managing food safety risks at different levels and stages (Pfeiffer, 2002).

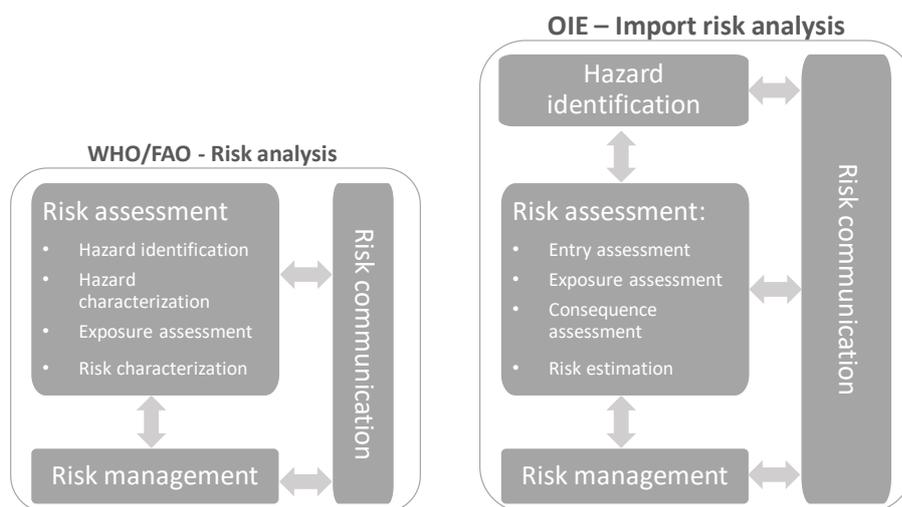


Figure 1: Components and steps in risk analysis frameworks proposed by WHO/FAO and OIE.

2.3.1 Mathematical modelling

Quantitative risk assessment usually involves the development of different types of models. Mathematical modelling helps us to better understand the epidemiology of the disease because the key causal relationships have to be presented in a structured manner (Dohoo et al., 2009). The

computerized simulations are particularly useful because of the lack of empirical data and when the actual experiment cannot be performed, either because of the cost, dangerous infectious diseases, ethical objections, or because of new untested research methods (Dijkhuizen and Morris, 1997). The principle of mathematical modelling is that a complex real system is simplified to such an extent that it can be systematically analysed and described by mathematical equations. Based on existing information, the models try to explain and predict the patterns of disease occurrence and to assess the likely impact of alternative strategies for disease control (Thrusfield, 2007). In veterinary epidemiology, the benefits of the mathematical and epidemiological modelling have long been recognized, nevertheless, they are still not exploited to their fullest potential as a decision support tool for optimizing animal health management (OECD, 2013).

When assessing the impact of paratuberculosis, its spread potential and the efficacy of different surveillance and control measures, problems such as lack of empirical data and scarce resources can be overcome to a certain extent by using different mathematical and computational methods. In case of paratuberculosis, these methods were used to study: (1) the disease dynamics at the herd level (Magombedze et al., 2013; Smith et al., 2015; Verdugo et al., 2018), (2) between-herd transmission and spread potential (Marquetoux et al., 2016a; Beaunée et al., 2015), (3) evaluation of the surveillance systems and control measures (Lu et al., 2010; Frössling et al., 2013; Beaunée et al., 2017), (4) assessment of economic consequences of the disease and control measures (Shephard et al., 2016; Kirkeby et al., 2016; Kirkeby et al., 2017; Smith et al., 2017; Verteramo Chiu et al., 2018), (5) MAP contamination of milk and (6) risk of human exposure to MAP via milk (Nauta et al., 1998; Cerf et al., 2007; Okura et al., 2013; Serraino et al., 2014; Rani et al., 2019). Network analysis of animal movements has proved to be particularly useful for studying the potential spread of disease among premises and a risk-based selection of premises that can serve as targets for surveillance and intervention measures (Frössling et al., 2012; Beaunée et al., 2015; Marquetoux et al., 2016b; VanderWaal et al., 2016). This way, informed surveillance and control measures are epidemiologically and economically more effective compared to non-targeted measures (Kiss et al., 2006; Natale et al., 2009; Mweu et al., 2013).

For our quantitative risk assessment the following data is required: herd level and within-herd level prevalence of MAP, the rate of shedding in milk and faeces by infected animals, the level of internal milk contamination (MAP excreted in milk) and external milk contamination (contamination of milk with MAP contaminated faeces or dirt), dilution effect on MAP concentration, the efficiency of pasteurisation in reducing the concentration of viable MAP and consumption of milk and milk products (Mihajlovic et al., 2011). We developed three different models, two to obtain the estimates on MAP prevalence in Slovenia which we then used for the final human exposure assessment model.

3 MATERIALS AND METHODS

To meet the objective of our study, we used a modified risk analysis framework outlined by WHO and FAO in the Risk Characterization of Microbiological Hazards in Food (WHO/FAO, 2009) combined with the guidelines of the World Organisation for Animal Health in the Handbook on Import Risk Analysis (Murray, 2004a; Murray, 2004b). We performed the first two components of the risk analysis, namely hazard identification and risk assessment, whilst risk management and risk communication were beyond the scope of this thesis, since by definition this is a task for decision makers. Our modified framework is a risk assessment framework divided into three steps. It is outlined in Figure 2 and each step is described in a separate section of this chapter.

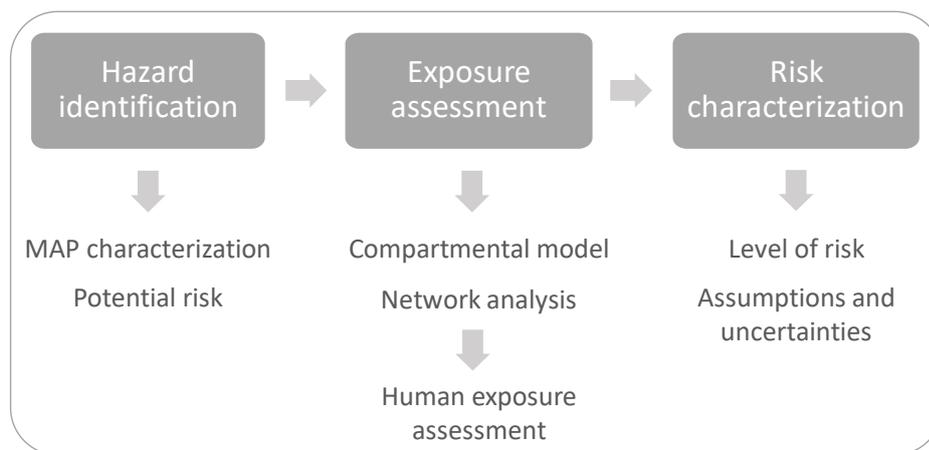


Figure 2: Framework for risk assessment of human exposure to MAP via milk and milk products.

3.1 HAZARD IDENTIFICATION

The first step in our risk assessment framework is *hazard identification*, where we reviewed the literature to collect MAP characteristics and epidemiological features that are important for modelling the disease spread and food contamination. We also examined the potential risks MAP may pose for human health.

3.2 EXPOSURE ASSESSMENT

The second step in our risk assessment framework is *exposure assessment*. We performed a quantitative risk assessment of human exposure to MAP via milk and milk products. We used different types of models to overcome the lack of empirical data and the problem of inadequate tests for diagnosing MAP infections. Modelling enabled the use of available data regarding the Slovenian cattle population, movements and production as well as the utilization of published data on MAP epidemiological features. Where necessary or deemed suitable, the data were supplemented with expert assessments.

We developed a compartmental model and a temporal network model to evaluate possible MAP spread in a typical Slovenian herd and between Slovenian herds based on cattle movement data. These two models were used as risk assessment tools and their main task was to obtain a possible epidemiological situation of MAP, specific for the Slovenian dairy industry. Their second purpose was to explore the Slovenian cattle trade network characteristics to determine if network analysis could

provide support in targeted paratuberculosis surveillance and control measures. Obtained results were used in the third model for human exposure assessment to MAP via milk and milk products. Below, we give a detailed description of these models.

3.2.1 Compartmental model

3.2.1.1 Model structure

We developed a stochastic compartmental model of within-herd MAP spread in a typical Slovenian dairy herd using the Epidemiological Multi-Level Simulation Framework (EMULSION), version 1.0.12 (Picault et al., 2019), which utilizes finite state machines classically used in computer science. In our model, the state machine is defined by the evolution of health states. In epidemiological modelling, these states are termed compartments. Transitions of individual animals between compartments are defined with different rates, which are automatically converted into probabilities thus introducing stochasticity.

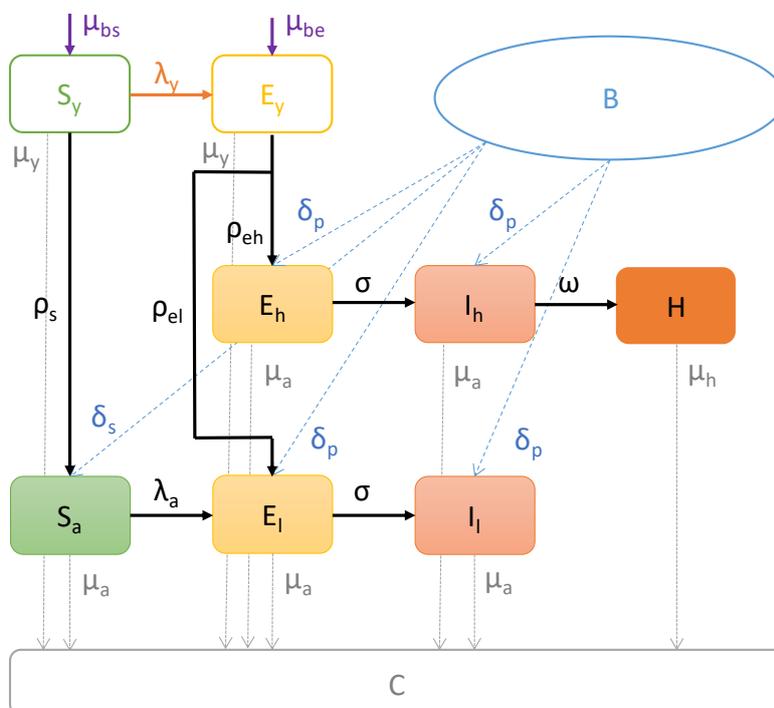


Figure 3: Flow diagram of the compartmental model for the within-herd spread of MAP in a typical Slovenian dairy herd. Possible health states are represented with boxes; these states are: susceptible (S_y) and latent young animals (E_y); latent cows (E_h), infected cows (I_h) and high shedding cows (H) on the high shedding path; susceptible cows (S_a); latent cows (E_l) and infected cows (I_l) on the low shedding path and culled, dead or sold animals (C). Compartment buying (B) represents animals outside the herd that can be bought in. Arrows represent rates at which individual animals move from one state to another; all parameters and calculations are defined in Tables 1 and 2.

To capture epidemiological features of MAP we used multiple compartments for adult cows and young animals. The basic structure of the model is shown as a flow diagram in Figure 3. Adult cows were divided into six possible health states (one susceptible and five MAP positive states) because we modelled two different paths of infection. Namely, the *low shedding path* with compartments

susceptible cows (S_a), *latent cows* (E_i) and *infected cows* (I_i) and the *high shedding path* with compartments *latent cows* (E_h), *infected cows* (I_h) and *high shedding cows* (H). Only animals infected as calves could enter the high shedding path, but they could also enter the low shedding path. Whereas, animals infected as adults could only enter the low shedding path. To model the effect on reproduction and infections in calves and heifers we added two compartments for young animals; *susceptible young animals* (S_y) and *latent young animals* (E_y). We considered infected cows on both paths as subclinically infected, and latent young animals and cows as animals not affected by any sign of disease. High shedding cows were considered to be clinically infected.

To capture culling or mortality of animals and the introduction of new animals into the herd we added two more compartments. Culled or dead animals were moved to the compartment *culled* (C) and were no longer counted, on the other hand the compartment *buying* (B) counted animals and initially held 1,000 animals. The latter represented animals outside the herd that could be bought and moved into the herd. It was created because EMULSION does not allow for the introduction of new individuals if the model is made on a compartmental level (this feature is available only for an individual-based model or hybrid models).

3.2.1.2 Parameters, model calibration and simulation

First, we constructed a basic model, wrote all transmissions between compartments and defined rates and if necessary, their calculations. Then we implemented parameter values for the average dairy herd in Slovenia based on data obtained from the Agricultural Institute of Slovenia (KIS), the Results of Dairy and Beef Recording and the Statistical Office of the Republic of Slovenia (SURS). All used data referred to the year 2018. The initial herd had 17 dairy cows (N_i) and 15 young animals (Y_i) in respective susceptible compartments. Parameters estimated based on Slovenian data were birth rate (μ_b), culling rates (μ_a, μ_h), renewal of the herd by own young animals (ρ_s) and by the introduction of new animals (δ) and animal-level paratuberculosis prevalence (P) for animals that can be introduced into the herd. We calibrated the model so that the herd size was stable for at least 20 years.

Parameters regarding MAP transmission, incubation, disease progression and birth rates of infected animals were obtained from published literature. These parameters were estimated either from the empirical data or through calibration of models similar to ours or other types of models developed by different authors, therefore we recalculated some values. First, we included the values that seemed agreed on by multiple authors. If that was not possible, we chose the value that corresponded to the worst-case scenario. Then we repeated the calibration of the model to make the herd size stable for at least 10 years. Based on expert opinion, we adjusted some parameter values. All parameter values in the model were deterministic. Parameter definitions, their values and sources are presented in Table 1. Calculations of parameters and transitions are shown in Table 2.

We simulated the within-herd spread of MAP for 10 years with 1,000 iterations and a daily time step. The infection started with the introduction of one infected animal into the naive herd, namely an infected cow on the high shedding path (I_h). Through the buying compartment, we accounted for possible reinfections. The rate of buying in animals (δ) was calculated so that each year there were approximately 3.1 cows introduced into the herd, which was based on cattle movement data, specifically on the average weighted in-degree per farm holding per year. The health state of a bought

animal depended on the animal-level prevalence (P). We assumed that bought animals were adults and could be of any health state except for high shedding cows. Therefore, for the definition of the transition rate from the buying compartment to other MAP positive compartments (δ_p), we divided the transition rate from the buying compartment between four MAP positive compartments. We estimated the animal-level prevalence from the results of two studies conducted in Slovenia.

Table 1: Parameters used in the compartmental model of the within-herd spread of MAP.

Symbol	Description	Value	Source
N_i	Initial number of adult animals; dairy cows	17	KIS
Y_i	Initial number of young animals; calves and heifers	15	KIS
σ	Transition rate from latent to infected cow (/day)	0.00274	Marcé et al., 2011a; Mitchell et al., 2015b
ω	Progression from infected cow on high shedding path to high shedding cow (/day)	0.00137	Marcé et al., 2011a; Mitchell et al., 2015b
β_e	Transmission rate for latently infected cow (/day)	0.000027	Robins et al., 2015; Al-Mamun et al., 2017
β_i	Transmission rate for infected cow (/day)	0.000274	Al-Mamun et al., 2017
β_h	Transmission rate for high shedding cow (/day)	0.00274	Al-Mamun et al., 2017
μ_a	Culling rate for all cows except high shedding cows (/day)	0.000457	Expert opinion
μ_h	Culling rate for high shedding cows (/day)	0.003653	Expert opinion
μ_r	Culling rate or sale of redundant young animals (/day)	0.000322	Expert opinion
δ	Rate of buying in new animals (/day)	0.000009	UVHVVR
P	Animal-level prevalence	0.08	Kušar et al., 2011; Logar et al., 2012
μ_b	Birth rate for all cows except high shedding cows (/day)	0.001315	Sadar et al., 2019
μ_{bh}	Birth rate for high shedding cows (/day)	0.000411	Smith et al., 2017
γ_p	Proportion of latent calves per latently infected and infected cows	0.25	Martcheva et al., 2015
γ_h	Proportion of latent calves per high shedding cows	0.65	Marcé et al., 2011a
β_{yy}	Transmission rate from latently infected calf to susceptible calf (/day)	0.000007	Al-Mamun et al., 2017
β_{ye}	Transmission rate from latently infected cow to susceptible calf (/day)	0.00021	Al-Mamun et al., 2017
β_{yi}	Transmission rate from infected cow to susceptible calf (/day)	0.002099	Al-Mamun et al., 2017
β_{yh}	Transmission rate from high shedding cow to susceptible calf (/day)	0.020986	Al-Mamun et al., 2017
β_{hh}	Transmission rate from latently infected heifer to susceptible heifer (/day)	0.000003	Al-Mamun et al., 2017
α	Proportion of latently infected young animals entering high shedding path	0.335	Smith et al., 2017

The rate of susceptible cows becoming latent cows on the low shedding path is defined by the force of infection (λ_a) which is dependent on the number of cows in each health state and different transmission potentials of cows in each state. Transmission rates for cows in different health states ($\beta_e, \beta_i, \beta_h$) were taken from the results of published models and recalculated to fit our model's time

step. Progression from a latently infected cow to an infected cow on the low and high shedding path is believed to take up to a year. In the model, this was defined with the rate of incubation (σ). In two years, infected cows on the high shedding path can progress to a high shedding cow that manifests clinical signs, which was defined with the rate of progression (ω). The culling rate of adult animals (μ_a) was derived from the assumption that in a typical Slovenian dairy herd cows reach six productive years. We assumed that farmers cull clinically affected animals within 0.75 years; defined with the culling rate of high shedding cows (μ_h). In the meantime, the culling rate of young animals (μ_y) is calculated so that the herd size stays stable throughout the simulation period. In our model, we name it culling of young animals, but in the real world, this could be either culling or mortality of young animals or even sale of redundant young animals.

Table 2: Calculation of parameters or transitions in the compartmental model of the within-herd spread of MAP.

Symbol	Description	Calculation
N	Number of adult animals; dairy cows	$N = S_a + E_l + E_h + I_l + I_h + H$
Y	Number of young animals; calves and heifers	$Y = S_y + E_y$
λ_a	Force of infection for adult animals (/day)	$\lambda_a = \frac{\beta_e(E_l + E_h) + \beta_i(I_l + I_h) + \beta_h H}{N}$
δ_s	Transition rate from buying to susceptible cow compartment	$\delta_s = \delta(1 - P)$
δ_p	Transition rate from buying to latently infected and infected cow compartments	$\delta_p = \frac{\delta \cdot P}{4}$
μ_{be}	Birth rate of latently infected female calves and direct transmission from mothers	$\mu_{be} = \mu_b S_a + \mu_b \gamma_p (E_l + E_h + I_l + I_h) + \mu_{bh} \gamma_h H$
μ_{bs}	Birth rate of susceptible female calves	$\mu_{bs} = \mu_b (1 - \gamma_p) (E_l + E_h + I_l + I_h) + \mu_{bh} (1 - \gamma_h) H$
λ_y	Force of infection for young animals (/day)	$\lambda_y = (\beta_{yy} + \beta_{hh}) \frac{E_y}{Y} + \frac{\beta_{ye}(E_l + E_h) + \beta_{yi}(I_l + I_h) + \beta_{yh} H}{N + \frac{Y}{2}}$
ρ_s	Replacement of culled animals with own animals; from S_y to S_a	$\rho_s = (N_i - N) \frac{2}{Y}$
ρ_{el}	Replacement of culled animals with own animals; from E_y to E_l	$\rho_{el} = \rho_s (1 - \alpha)$
ρ_{eh}	Replacement of culled animals with own animals; from E_y to E_h	$\rho_{eh} = \rho_s \cdot \alpha$
μ_y	Culling or sale of young animals (/day)	$\mu_y = \begin{cases} \mu_r, & \text{if } Y - Y_i > 0 \\ 0, & \text{otherwise} \end{cases}$

Renewal of the herd was considered by adding the buying compartment as well as replacement from own young stock. Young animals were born with the rate depending on the birth rate of female calves per cow (μ_b). We assumed that only the high shedding cows have a lower birth rate (μ_{bh}). Calves could be born susceptible (S_y) or already latently infected (E_y). The probability of calves born infected and calves directly infected from their mothers after birth was different for latently infected cows and infected cows (γ_p) on the low and high shedding path and for the high shedding cows (γ_h). Additionally,

susceptible young animals could get infected from all other animals in the herd; which is taken into account with the force of infection for young animals (λ_y). Since we modelled calves and heifers together as young animals, we calculated their force of infection assuming that each of them represented half of the young animals in the herd. Progression of young animals to adult animals was calculated so that the herd size stayed stable. Susceptible young animals became susceptible cows, whereas latently infected young animals progressed to latently infected cows on the low or high shedding path. The proportion of latent young animals entering the high shedding path was determined with α .

3.2.1.3 Model outputs and validation

The last step in the model development was internal validation, which consisted of a thorough examination of all underlying assumptions, calculations and parameters.

Since the motivation for modelling the within-herd prevalence was in producing the data for the human exposure to MAP, we only calculated results concerning cows. The outputs of our interest were the number of dairy cows, prevalence and number of subclinically and clinically infected cows. Prevalence accounted for all infected dairy cows in five different compartments, whereas subclinically infected animals were those in infected compartments on the low and high shedding paths and clinically infected animals were those represented with the high shedding compartment.

In addition, we performed a sensitivity analysis on the epidemiologically most important parameters with minimum and maximum values as estimated by the expert to assess the robustness of the model. We rerun the model for 16 times with all parameters fixed except for the one that was tested. The values used in the sensitivity analysis are shown in Table 3. The maximum value for transition rate from a latent to an infected cow (σ) and progression from an infected to a high shedding cow (ω) are smaller than the values used in the model because they are calculated based on the expert opinion on how long the individual animal may stay in the latent or infected compartment. To be specific, the expert estimated that a cow may stay latently infected for a maximum of three to four years and infected for up to five years. The minimum value of zero for these two parameters means that a cow will never progress to an infected or a high shedding state, respectively. We analysed the results obtained by EMULSION with the R programming language, version 3.5.0 (R Core Team, 2018).

Table 3: Minimum and maximum values for the epidemiologically most important parameters used in the sensitivity analysis.

Parameter	Value used in the basic model	Minimum value	Maximum value
σ	0.00274	0	0.000685
ω	0.00137	0	0.000548
β_i	0.000274	0	0.00274
β_h	0.00274	0	0.027398
P	0.08	0.01	0.2
β_{yi}	0.002099	0	0.013699
β_{yh}	0.020986	0.00274	0.068494
α	0.335	0.1	0.5

3.2.2 Network analysis

3.2.2.1 Movement data

The data on cattle movements from 1st January 2010 to 5th August 2016 were obtained from the UVHVVR of the Ministry of Agriculture, Forestry and Food. The UVHVVR holds the data of the life history events of each individual bovine animal including all movements between all types of premises within Slovenia and with import or export countries in the central register of bovine animals. Data provided by the government were stored in two datasets. In the first dataset, each line denoted the movement of an individual animal on a particular date, namely: country of origin, animal ID, source premises, target premises, date of movement, date of birth, sex, breed, type of movement and import or export country. The second dataset included premises ID, municipality, type of premises and geographic coordinates.

We extracted the information on the movements for the last five available years, namely data from 1st August 2011 to 31st July 2016. As we were interested in movements that are epidemiologically important for the spread of disease, we omitted all movements to premises that corresponded to end nodes. These are premises that animals never leave, at least not alive. Therefore, we excluded movements to slaughterhouses, animal waste processing centres and corpse collection centres. For export movements, the use of animals at the target destination was not included in the dataset, consequently, the export movements to end nodes remained in the data. Included types of premises were farm holdings, pastures, mountain pastures, collection centres, exhibitions and fairs. Collection centres are registered facilities where animals from different premises are grouped together intended for movements in consignments, whether for transfer, trade, import or export. Similarly, fairs are also registered facilities, but animals in consignments are intended only for trade (Rules on the conditions to be met by the assembly centre operators and retailers with animals, 2006). Exhibitions are events where animals are usually not grouped together and trade is not the primary aim. At the collection centres, exhibitions and fairs animals stay only for a day or a couple of days. At the pastures and mountain pastures, they usually spend the whole pastoral season. In Slovenia, the pastoral season starts at the beginning of April and lasts until the middle of November.

For the 345 premises with missing information on the type of premises, we performed an individual search in the VOLOS portal¹, where the central register of bovine animals is accessible. For the remaining 27 premises with an unknown type, we analysed the cumulative trade volume as described in Koeppel et al. (2018) and assigned the type of premises accordingly.

We believe that even though unrecorded movements may have been present, they did not hinder the validity of the represented cattle trade network considerably. In Slovenia, bovine animals must be marked as soon as possible, mandatorily before the first movement, but the latest until the age of 20 days, and all movements must be registered (Pravilnik o identifikaciji in registraciji goveda, 2016). In the analysis, we used the national data and based on the proportion of active premises, we assume

¹ <https://www.gov.si/zbirke/storitve/dostop-do-spletnega-portala-volos/>

that the large majority of movements are indeed registered. For example, in 2013 28,284 farm holdings were involved in cattle trade, according to census data this was 82.98% of farm holdings with cattle².

3.2.2.2 *Static network analysis*

The final trade dataset consisted of source and target premises, date of movement and batch size. Only premises involved in animal trade during the five-year study period were included in the analysis. To get a tangible overview of the cattle trade network structure, we considered two time windows and constructed sixty monthly and five yearly static networks. Networks of premises (nodes) connected through cattle movements (arcs) were generated in the Pajek software, version 5.06 (Batagelj and Mrvar, 2018). In each of these networks, we aggregated individual movements between the same two premises into batches when the direction of movements coincided. Networks were directed (movement is directed to premises receiving the animal) and weighted (the number of moved animals between pairs of premises varied). Nodes had an assigned type of premises. Network analysis was performed using the Pajek software and the igraph package in the R programming language. Statistical analysis and graphs were also performed in the R programming language. Premises were regarded as active if they participated in at least one animal movement in the specified period. Import and export countries appear as single nodes in the dataset and were thus excluded from the calculation of network measures.

In order for the animal movements to be of appropriate assistance to the informed risk-based targeted surveillance and control measures, the network of movements should exhibit three key characteristics. First, it has to show some consistency of properties over time. Second, premises ought to be of heterogeneous importance to the overall connectivity of the network. Third, associated with the latter, the structure of the network should follow some order. In other words, the structure should not have emerged only by chance. We explored the characteristics of cattle movement networks through global measures, namely by the number of active premises, sizes of giant weakly and strongly connected components (GWCC and GSCC, respectively), bow-tie partition, density, average shortest path length, diameter, average degree, clustering coefficient and degree assortativity (de Nooy et al., 2005; Mrvar and Batagelj, 2018). In local network measures or node centrality measures, we focused on measures that were recognized as important for disease transmission in previously published studies (Kiss et al., 2006; Ortiz-Pelaez et al., 2006; Natale et al., 2009; Volkova et al., 2010; Nöremark et al., 2011; Marquetoux et al., 2016b; VanderWaal et al., 2016; Salines et al., 2018; Vidondo et al., 2018). We calculated the unweighted and weighted total, in- and out-degree, betweenness centrality and total, in- and out-degree closeness centrality (de Nooy et al., 2005). The explanation of the above-mentioned global and local network measures is shown in Table 4.

² https://pxweb.stat.si/SiStatDb/pxweb/en/30_Okolje/30_Okolje__15_kmetijstvo_ribistvo__03_kmetijska_gospod__02_15166_zivinoreja/?tablelist=true

Table 4: Definitions of network measures used in the static network analysis.

Network measures	Definition
Global network measures	
<i>Giant weakly connected component (GWCC)</i>	A set of nodes that are connected to each other by path of any length ignoring the direction of arcs (de Nooy et al., 2005).
<i>Giant strongly connected component (GSCC)</i>	A set of nodes where there is a path of any length between any node pair when accounting for the direction of arcs (de Nooy et al., 2005).
<i>Bow-tie partition (Figure 12A)</i>	Partition of nodes in a directed network that assigns nodes to the following components (Broder et al., 2000; Mrvar and Batagelj, 2018): <ul style="list-style-type: none"> • GSCC, • <i>giant in component</i> (GIC) – nodes in this component can reach GSCC, but there are no arcs leading from GSCC to GIC, • <i>giant out component</i> (GOC) – nodes in this component can be reached from GSCC, but there are no arcs leading back to GSCC, • <i>tendrils</i> – nodes in tendrils cannot reach GSCC and cannot be reached from GSCC, arcs are leading away from GIC and into GOC, • <i>tubes</i> – are tendrils connecting GIC and GOC without contact with GSCC, • <i>others</i> – all other nodes. A set of nodes not connected to GWCC.
<i>Density</i>	A proportion of the maximum possible number of arcs in the network that are realized in the given network (de Nooy et al., 2005).
<i>Average shortest path length</i>	An average distance (the number of arcs) between all node pairs in the network (de Nooy et al., 2005).
<i>Diameter</i>	The length of the longest shortest path in the network between a pair of nodes in GWCC (de Nooy et al., 2005).
<i>Average degree</i>	An average number of arcs per node in the network (de Nooy et al., 2005).
<i>Clustering coefficient</i>	An average value of node level clustering coefficients, which show a probability that two nodes connected to a third node are also connected to each other (Kiss et al., 2006).
<i>Degree assortativity</i>	A measure of the tendency of nodes to connect with nodes with similar degrees (Kiss et al., 2006).
Local network measures/Node centrality measures	
<i>Unweighted total, in- and out-degree</i>	Total unweighted degree of a node is a sum of the number of nodes that can reach a specified node (in-degree) and the number of nodes that can be reached from this particular node (out-degree). Arcs are aggregated if they connect the same two nodes premises and have the same direction (de Nooy et al., 2005).
<i>Weighted total, in- and out-degree</i>	Total weighted degree of a node is a sum of the number of arcs leading from nodes that can reach a specified node (in-degree) and the number of arcs leading to nodes that can be reached from this particular node (out-degree) (de Nooy et al., 2005).
<i>Betweenness centrality</i>	A measure of a specified node's control over the communication in the network. If the betweenness is large, it means that most of the shortest paths are passing through this node (de Nooy et al., 2005).
<i>Total, in- and out-degree closeness centrality</i>	Closeness of a node is the reciprocal average distance to all other nodes that can be reached from a specified node (de Nooy et al., 2005).

We evaluated the consistency of global and local network measures at monthly and yearly resolutions. Using the Mann-Kendall test, we investigated whether there was any trend in the network measures on a monthly resolution. Correlations of node level measures across years and between different measures were assessed with Spearman's rank correlation coefficients (VanderWaal et al., 2016; Marquetoux et al., 2016b; Ortiz-Pelaez et al., 2006; Vidondo et al., 2018). We assessed the heterogeneity of premises contributions to the overall network connectivity utilizing degree distributions (de Nooy et al., 2005). Since the degree distributions are heavy-tailed, we plotted yearly and monthly distributions using a logarithmic scale. The number of contacts contributed by nodes with the highest degrees was calculated. We tested for the differences in centrality measures between different types of premises using the Kruskal-Wallis rank sum test and post hoc multiple comparison test (Ortiz-Pelaez et al., 2006; Marquetoux et al., 2016b). To test whether the features of the Slovenian cattle movement networks could have occurred simply by chance, we compared the average path length, diameter, clustering coefficient, assortativity, size of GWCC and GSCC of our yearly networks to 1,000 random networks (Erdős-Rényi networks) of the same size and density (Natale et al., 2009; Marquetoux et al., 2016b; VanderWaal et al., 2016).

Since local network measures give information on the relative importance of each node in the network, we can use them to simulate targeted intervention measures. In network analysis, this for example means removing nodes with the highest centralities from the network (Kiss et al., 2006; Lentz et al., 2016; Marquetoux et al., 2016b). In practice, this could be implemented as targeted surveillance, movement restriction, isolation, culling, vaccination or increased biosecurity measures. Due to the fact that decisions on future intervention measures must be made based on the currently available data, values from previous years were used to rank nodes. Nodes were sorted in decreasing order by a precalculated selected measure and then removed one by one. We simulated targeted intervention measures by removing 400 nodes from the fifth yearly network based on total degree, betweenness and closeness centrality from the same year and from previous years. At each step, the size of the GSCC for the remaining network was calculated, since the behaviour of the GSCC mimics the functionality of the network. In addition, we simulated the removal of nodes by random ordering.

3.2.2.3 SI model on the temporal network

So far, the considered system was treated as static, which means that the temporal nature of cattle movements was neglected. Since all cattle movements are provided with timestamps, it is possible to simulate a potential disease spread on the network explicitly over time. This circumstance cannot be addressed using standard network analysis; therefore, temporal network analysis has emerged over the last years (Lentz, 2013; Lentz et al., 2016). A static network can be represented as a list of arcs e , where each element contains a contact between nodes. As an example, the list of arcs $e = [(1, 2), (3, 4)]$ would represent a network, where there is an arc from node one to node two and an arc from node three to node four. In temporal networks, trade contacts are time-stamped and are represented as a list of triples, e.g. $e_{temporal} = [(1, 2, 1), (3, 4, 2), (1, 2, 3)]$ means that the contact from node one to node two was active at times one and three and the contact from node three to node four was active at time two. In order to distinguish contacts between nodes in temporal networks from static ones, we call a temporal contact from node i to j at time t (i, j, t) an edge, whereas there is an arc (i, j) between i and j in the static network, when there was at least one edge in the studied period.

To assess the possible spread of MAP through cattle movements in the studied period, we simulated the spread of disease using a susceptible-infectious (SI) model using the temporal trade data explicitly. The analysis was carried out using the Python programming language, version 3.7.3 (Python Software Foundation, 2019). We adopted the model developed by Lentz et al. (2013). The original SI model was developed to take into account causality by considering the succession of movements and thus it provides the so-called unfolding of the accessibility graph of a temporal network. This is also known as network reachability or out-going contact chain (Nöremark et al., 2011). Compared to other methods, the method used here computes the out-going infection chain of all nodes at the same time efficiently. It thus generates the whole reachability matrix in a single run. The original model describes a possible spread of disease if the probability of transmission is equal to one. For a detailed description of the model, see Lentz et al. (2013; 2016) and the developed Python code³.

In order to simulate the spread of MAP on our studied network, the possibility of applying a probability of transmission with values less than one had to be implemented. Therefore, we improved the model of Lentz et al. (2016). Different transmission probabilities were realized with a dilution of the initial studied temporal network where an edge represented the movement of an individual animal (Figure 4). This means that the probability of infection per movement was modelled by removing a certain number of edges from the whole network. Statistically speaking, we replaced the probability of transmission by another probability, namely the probability of contact. More precisely, if $p = \beta \cdot \tau$ is the probability of transmission between two nodes (where β is the infection probability given contact and τ is the contact probability), we set β to one and vary τ in order to adjust p . The dilution of the network was carried out by a random sampling of edges from the whole temporal network based on the value of the transmission probability we wanted to simulate. To be specific, the proportion of edges in the diluted network was equal to the chosen probability of transmission. Since the edges were kept in the diluted network based on a random sampling and a deterministic value for the probability of transmission was used, we adopted two limiting assumptions underlying the implemented SI model. First, a within-herd prevalence is assumed to be the same in all infected herds and second, when an infected animal is moved, the receiving herd always becomes infected. Therefore, in this case, the probability of transmission can also be described as the within-herd prevalence or the probability that the moved animal is diseased. This model can be used to study the between farm spread of any disease that primarily spreads with animal movements. Here, we used the initial conditions and parameter values corresponding to the latest available data on paratuberculosis within-herd and herd prevalence in Slovenia.

³ <https://github.com/hartmutlentz/TemporalNetworkAccessibility>

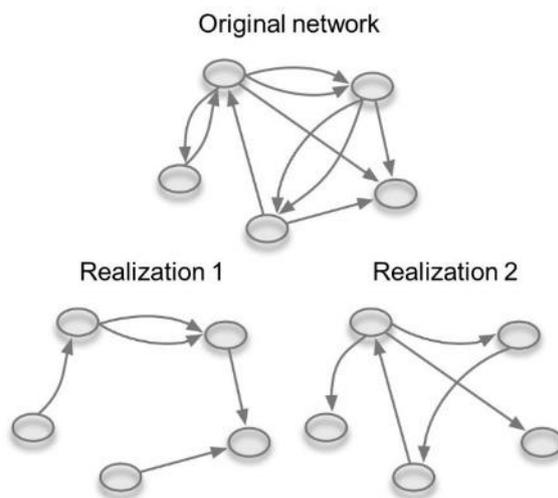


Figure 4: Dilution of the temporal network. Based on the value of the probability of transmission (p) random edges are omitted from the network. Two realizations of the same network for $p = 0.5$ are shown.

We used the temporal network with the full available time resolution (1 day) as a substrate for paratuberculosis spread and we tracked the spread between premises for 1826 days. We evaluated the influence on paratuberculosis spread of four different values for the probability of transmission (p). The estimated scenario ($p = 0.25$) reflects the results of a study of within-herd prevalence conducted in 2010, where they estimated prevalence in three different herds in Slovenia based on four combinations of three diagnostic methods and three different samples (Logar et al., 2012). Since the study included only three herds, significant uncertainty is associated with the estimate. For this reason, additional scenarios were included based on estimations of the within-herd prevalence from the literature; namely: best-case scenario ($p = 0.02$), middle scenario ($p = 0.15$) and worst-case scenario ($p = 0.40$) (Beaunée et al., 2015). We prepared one hundred diluted networks for each scenario.

Next, we selected the initially infected premises by random sampling among all active farm holdings in the studied period. In 2008, the true prevalence of paratuberculosis in Slovenia was estimated at 18.49% (Kušar et al., 2011); accordingly, we selected 6,818 farm holdings as initially infected. For each diluted network, we performed ten simulations of the SI model with randomly selected initially infected farms according to the estimated prevalence. Even though we sampled only from farm holdings, we kept all types of premises in the network. Consequently, we allowed for the transmission of disease, even if a pair of premises was not directly connected, but rather connected via a mountain pasture or a fair for instance.

Using the above-mentioned network dilution and initial conditions, we simulated the SI-infection spreading for 100 diluted networks and ten realizations of initial conditions for each probability of transmission, yielding 1,000 simulations per transmission probability. The output of the model was the number of infected farm holdings at each time step, i.e. 1,000 infection curves for each transmission probability. In addition, we calculated the slopes of all infection curves in order to highlight significant incidence changes.

3.2.3 Human exposure assessment

3.2.3.1 Model structure and parameters

To estimate the potential human exposure to MAP via milk and milk products in Slovenia we performed a stochastic quantitative risk assessment with Monte Carlo simulations. We focused on products or types of milk that were shown to be more important for human exposure in previous studies and were more relevant due to production and consumption trends in Slovenia. Nevertheless, we were constrained with the lack of data on MAP persistence during milk processing. Same as in the network analysis of cattle movements, we did not consider the import or export of raw and processed milk and milk products, since studying the dairy industry of importing countries was beyond the scope of this thesis.

In this model, we used the results from the compartmental model and temporal network model, available data on the dairy industry situation in Slovenia and data from the literature. We obtained and analysed the data from SURS, KIS, Agency of the Republic of Slovenia for Agricultural Markets and Rural Development and Chamber of Commerce and Industry of Slovenia. All data used referred to the last available year, which is to the year 2018. We fitted distributions to the data on the number of dairy cows per herd, milk yield per cow in Slovenia and the results of our previous two models and selected distributions with the minimum Akaike Information Criterion (AIC) value. There are different statistics available for selecting the best fitted distribution for the given data. We chose AIC because it takes into account the log-likelihood function as well as the number of estimated parameters for selected distribution (Palisade Corporation, 2016). Distributions for other parameters were taken from the literature and some were complemented or estimated based on expert opinion. All probability distributions with their values for input variables are shown in Table 5.

Our human exposure assessment model has three parts: a farm level model, a dairy industry level model and a possible human exposure model. The schematic structure of the model and types of milk considered are presented in Figure 5.

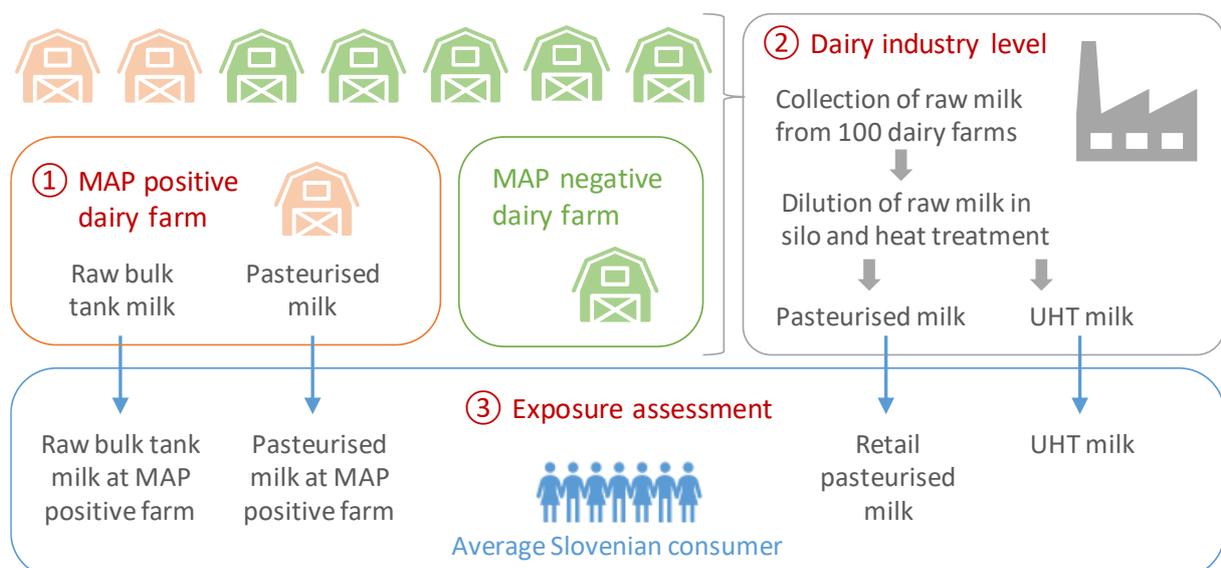


Figure 5: Schematic structure of the human exposure assessment model.

Table 5: Probability distributions of input variables used in the model of human exposure assessment to MAP by different types of milk.

Symbol	Description	Probability distribution	Source
Herd level prevalence (S_i)			
S_1	Herd level prevalence, best-case scenario (%)	$S_1 = Normal(21.2991, 0.10283)$	Temporal network model
S_2	Herd level prevalence, middle scenario (%)	$S_2 = Normal(38.88523, 0.31328)$	Temporal network model
S_3	Herd level prevalence, estimated scenario (%)	$S_3 = Normal(49.21046, 0.32587)$	Temporal network model
Within-herd level prevalence			
N	Number of dairy cows per herd	$N = Pearson6(2.2799, 3.8476, 21.026)$	KIS
P_s	Proportion of subclinically infected cows	$P_s = \begin{cases} Expon(0.064147, Truncate(0, 0.5)), & \text{if } N < 50 \\ Triang(0.051, 0.15, 0.51), & \text{otherwise} \end{cases}$	Compartmental model; Nauta et al., 1998; Rani et al., 2019; expert opinion
P_c	Proportion of clinically infected cows	$P_c = \begin{cases} Expon(0.0075005, Truncate(0, 0.1875)), & \text{if } N < 50 \\ Triang(0.009, 0.018, 0.022), & \text{otherwise} \end{cases}$	Compartmental model; Nauta et al., 1998; Rani et al., 2019; expert opinion
Milk production			
m_h	Milk production per healthy cow (litres/day)	$m_h = Lognorm(25.503, 5.1687, Shift(-4.9786), Truncate(15, 60))$	KIS
η_s	Proportion of milk produced per subclinical cow	$\eta_s = Triang(0.88, 0.94, 0.96)$	Nielsen et al., 2006; Raizman et al., 2007; Beaunée et al., 2015; Rani et al., 2019; expert opinion
η_c	Proportion of milk produced per subclinical cow	$\eta_c = Triang(0.35, 0.75, 0.85)$	Bates et al., 2018; expert opinion
Milk contamination with MAP			
MAP_{ms}	MAP shedding in milk per subclinical cow (log CFU/litre)	$MAP_{ms} = Triang(0, \log_{10} 5 \times 10^4, \log_{10} 8.8 \times 10^4)$	Sweeney et al., 1992; Beaunée et al., 2015; Rani et al., 2019; expert opinion
MAP_{mc}	MAP shedding in milk per clinical cow (log CFU/litre)	$MAP_{mc} = Triang(0, \log_{10}(5 \times 10^4), \log_{10}(8.8 \times 10^4), Truncate(1.6, \log_{10}(8.8 \times 10^4)))$	Giese et al., 2000; Beaunée et al., 2015; Rani et al., 2019; expert opinion
MAP_{fs}	MAP from faeces per subclinical cow (log CFU/litre)	$MAP_{fs} = \log_{10}(1 + 10^3 \cdot Beta(1, 25))$	Beaunée et al., 2015
MAP_{fc}	MAP from faeces per clinical cow (log CFU/litre)	$MAP_{fc} = \log_{10}(10^{3+10 \cdot Beta(50, 200)})$	Beaunée et al., 2015
φ	Milk filter efficiency	$\varphi = Uniform(0, 0.67)$	Van Kessel et al., 2008; Rani et al., 2019; expert opinion
ε	MAP reduction with pasteurisation (log CFU)	$\varepsilon = Uniform(4, 7)$	Serraino et al., 2014

The farm level model was used to estimate the level of contamination of bulk tank milk at a MAP positive farm. The number of cows per herd was simulated from Pearson type VI distribution with an average of 17 cows per herd (N). Then the number of healthy, subclinical and clinical cows for this herd size was calculated. Since the dynamics of the within-herd spread of MAP differs between small and big herds, we included two options for calculating the proportion of clinical and subclinical cows (P_s, P_c) based on expert opinion. The chosen cut-off value for what constitutes for a small herd was 50 cows. Consequently, if the model simulated less than 50 cows per herd, we used the results from our compartmental model, but if 50 or more animals were simulated, we assumed distribution based on published data and expert opinion.

The amount of milk produced per healthy cow (m_h) was simulated from a lognormal distribution with an average of 20.59 litres of milk per cow per day. The reduction in milk production per subclinical (m_s) and clinical cows (m_c) was assumed and estimated based on published literature and expert opinion. The triangular distributions for MAP shedding in milk by subclinical (MAP_{ms}) and clinical cows (MAP_{mc}) were defined by the expert based on published estimates. Whereas, the distributions for milk contamination with MAP through faecal contamination by subclinical (MAP_{fs}) and clinical cows (MAP_{fc}) were taken from the literature. Internal milk contamination (MAP_{int}) and external milk contamination (MAP_{ext}) were then calculated based on milk production at the herd level (m). Final MAP contamination of raw bulk tank milk (MAP_{herd}) was reduced up to 67% by filtration of milk (φ). Calculations are shown in Table 6.

The second part of the exposure assessment model was the dairy industry level model where we modelled the dilution of MAP in milk silo. Since we did not have information on which farms deliver milk to the same dairy plant, we assumed a random selection of farms and therefore, we were not concerned with the size of the silo. However, we calculated the number of farms for our simulation based on a silo with a capacity of 30,000 litres. Additionally, we assumed that farms that deliver milk to the dairy plant have at least three dairy cows. We simulated the collection of milk in a silo using one hundred farm level models ($MAP_{industry}$). For determining the herd infection status (HS) of each simulated farm we used fitted distributions of herd level prevalences from the temporal network model (S_i). However, we neglected the worst-case scenario as it was deemed highly unlikely that more than 50% of herds in Slovenia were MAP positive. The model assumed a random distribution of positive herds.

The third part of the exposure assessment model combines results from the first two parts with the data on MAP reduction during processing and consumption in order to model MAP contamination of milk at the farm and dairy industry level and thus possible human exposure. We simulated MAP contamination of raw milk and pasteurised whole milk for positive farms and at the dairy industry level for the afore-mentioned three scenarios of herd level prevalences. For the pasteurisation process, the reduction of MAP for at least 4 to 7 log colony forming units (CFU)⁴ was used (ε). Random process of MAP cells distribution in bulk tank milk ($MAP_{herd\ raw}, MAP_{herd\ past}$) or silo ($MAP_{industry\ past}$) was accounted for by using Poisson distribution.

⁴ The terms MAP CFU and MAP cell can be used interchangeably. We used the term MAP CFU as the unit of detection of viable MAP, while the term MAP cell referred to the actual quantity of individual MAP.

The results of this model are expressed as MAP contamination of different types of milk with the distribution of MAP cells per litre, proportions of MAP positive litres of milk or litres of milk containing > 0 MAP cells and proportions of litres of milk containing > 100 MAP cells per litre. These values were chosen based on previous studies (Serraino et al., 2014) and do not reflect the risk that MAP may pose for human health since the infectious dose for humans (assuming MAP is a zoonosis) is not known.

Table 6: Calculations used in the model of human exposure assessment to MAP by different types of milk.

Symbol	Description	Calculation
HS	Herd status	$HS = \text{Bernoulli}(S_i)$
N_s	Number of subclinically infected cows	$N_s = \begin{cases} P_s \cdot N, & \text{if } P_s \cdot N + N_c > 0 \\ 1, & \text{otherwise} \end{cases}$
N_c	Number of clinically infected cows	$N_c = P_c \cdot N$
N_h	Number of healthy cows	$N_h = N - N_s - N_c$
Milk production		
m_s	Milk per subclinically infected cow (litres/day)	$m_s = m_h \cdot \eta_s$
m_c	Milk per clinically infected cow (litres/day)	$m_c = m_h \cdot \eta_c$
m	Milk per herd (litres/day)	$m = \sum_{i=1}^{N_h} m_{h,i} + \sum_{j=1}^{N_s} m_{s,j} + \sum_{k=1}^{N_c} m_{c,k}$
Milk contamination with MAP		
MAP_{int}	Internal milk contamination (log CFU)	$MAP_{int} = \sum_{j=1}^{N_s} m_{s,j} \cdot MAP_{ms} + \sum_{k=1}^{N_c} m_{c,k} \cdot MAP_{mc}$
MAP_{ext}	External milk contamination (log CFU)	$MAP_{ext} = \sum_{j=1}^{N_s} m_{s,j} \cdot MAP_{fs} + \sum_{k=1}^{N_c} m_{c,k} \cdot MAP_{fc}$
MAP_{herd}	Bulk tank raw milk contamination on farm level (log CFU)	$MAP_{herd} = \left(\frac{MAP_{int} + MAP_{ext}}{m} \right) \cdot (1 - \varphi)$
$MAP_{herd\ raw}$	Raw milk contamination on farm level (CFU/litre)	$MAP_{herd\ raw} = \text{Poisson}(10^{MAP_{herd}})$
$MAP_{herd\ past}$	Pasteurised whole milk contamination on farm level (CFU/litre)	$MAP_{herd\ past} = \text{Poisson}(10^{MAP_{herd} - \varepsilon})$
$MAP_{industry}$	Silo milk contamination on dairy industry level (log CFU)	$MAP_{industry} = \sum_{i=1}^{100} MAP_{herd,i}$
$MAP_{industry\ past}$	Pasteurised whole milk contamination on dairy industry level (CFU/litre)	$MAP_{industry\ past} = \text{Poisson}(10^{MAP_{industry} - \varepsilon})$

Finally, we calculated the yearly exposure of an average Slovenian consumer who consumed 43 litres of milk in 2018. Since the consumption is given for all types of milk together, we calculated the average yearly consumption of different types of milk based on shares on the market, assuming that the average consumer consumes all these types of milk. According to SURS data for 2018 the consumption

of raw milk on farms and the direct sale of raw milk amounted to more than 13 million litres of milk. In the same year, the Chamber of Commerce and Industry of Slovenia recorded that dairy plants produced more than 16 million litres of pasteurised milk and almost 150 million litres of ultra-high temperature treated (UHT) milk.

3.2.3.2 Model simulation and sensitivity analysis

We performed 10,000 iterations of Monte Carlo simulations with Latin hypercube sampling using @RISK programme, version 8.0 (Palisade Corporation, 2019). Monte Carlo simulations are repeated calculations of our model with each iteration relying on random sampling from defined probability distributions of input variables. For sampling method, we chose the Latin hypercube sampling which forces sampling from all parts of the distribution. This is stratified sampling, meaning that each probability distribution of input variables is divided into equal parts or strata. Random sampling is then performed in each stratum, whereas in traditional Monte Carlo sampling a random sampling is performed directly from probability distributions. Therefore, to achieve the same level of accuracy the Latin hypercube sampling requires fewer iterations of simulations than the Monte Carlo sampling method (Palisade Corporation, 2016).

Inputs with the greatest impact on the outputs of the model were identified by sensitivity analysis. The results of the sensitivity analysis show the sensitivity of the output variable to the distribution of each input in the model. Additionally, we tested the influence of different cut-off values for the small herd size.

3.3 RISK CHARACTERIZATION

The last step in our risk assessment framework is *risk characterization*, where the results of *exposure assessment* are summarized and presented with qualitative terms based on the terminology proposed by WHO/FAO (2009) and EFSA (2017). For the qualitative expression of the likelihood of human exposure to MAP we used the terms: *insignificant* – not expected to occur, *rare* – may occur only in exceptional circumstances, *unlikely* – could occur at some time, *possible* – might occur or should occur at some time, *likely* – will probably occur in most circumstances, and *almost certain* – expected to occur in most circumstances. We defined the impact or consequences in terms of average concentration of MAP per litre of milk using the terms: *insignificant* (0 MAP CFU/litre), *low* (1–10 MAP CFU/litre), *moderate* (11–100 MAP CFU/litre) and *high* (> 100 MAP CFU/litre). The level of risk was expressed with *negligible*, *low*, *moderate*, *high* and *very high*, depending on the determined likelihood of human exposure and average concentration of MAP per litre of milk. We reported the level of risk for each assessed type of milk separately and the overall risk for the average Slovenian consumer.

Furthermore, we identified used assumptions and uncertainties and discussed their impact on the results as well as the interpretation of our findings. We also expressed the level of uncertainty with the qualitative terms *low*, *moderate* or *high*, meaning that there were sufficient, some or few scientific evidence available to support the assumptions and outcomes of our models, respectively.

4 RESULTS

4.1 HAZARD IDENTIFICATION

4.1.1 MAP characterization

MAP is a small ($0.5 \times 1.5 \mu\text{m}$), rod shaped, Gram positive, acid-fast and facultative anaerobic bacterium with a thick lipid-rich cell wall (Lambrecht et al., 1988). This thick waxy cell wall renders MAP acid-fast, hydrophobic and highly resistant to chemical and heat treatment (Verma, 2013). In contrast to other mycobacteria, MAP is not able to synthesise mycobactin – an iron-binding siderophore that is required for growth; therefore, it has to be added to the cultivation media (Francis et al., 1953; Snow, 1965). The generation time of MAP is over 20 hours (Lambrecht et al., 1988), which makes MAP the slowest growing bacteria from its genus (Gill et al., 2011). Besides its characteristic slow growth, it is known to have a tendency to form clumps (Rowe et al., 2000).

MAP is an obligate intracellular pathogen and is believed to be unable to multiply outside of the animal host. Next to the gastrointestinal tract, the bacteria can be detected in different tissues during systemic infection. In cattle, MAP has been found in many different tissues and internal organs; e.g. blood, lung, liver, spleen, kidney, uterus, mammary gland, epididymis, lymph nodes and muscles (Collins, 1997; Alonso-Hearn et al., 2009; Gill et al., 2011; Mihajlovic et al., 2011;). MAP can infect lymph nodes associated with the mammary gland and be shed in the colostrum and milk by cows with clinical signs as well as by asymptomatic animals. The amount of MAP shed in milk and colostrum depends on the stage of the infection and of lactation (Stabel, 2008). MAP was also found in semen (Ayele et al., 2004).

The main source of environmental contamination and consequently the spread of the pathogen is faecal shedding by cattle. The amount of MAP shed in faeces depends on the stage of the infection (Sweeney, 2011). Based on the amount of MAP in faeces animals are defined as low ($< 300 \text{ CFU/g}$), moderate ($300\text{--}3000 \text{ CFU/g}$) and heavy shedders ($> 3000 \text{ CFU/g}$) (van Schaik et al., 2003c). Animals in the latent period, which is usually long, can already shed small quantities of MAP in faeces and even more in the subclinical phase (Benedictus et al., 2008), while clinically infected animals can excrete up to 10^9 CFU per gram of faeces (van Roermund et al., 2004).

Contaminated faeces contributes to the contamination of the immediate and broader environment. Different studies showed that in a dairy herd the extent of environmental contamination with MAP was positively correlated with the prevalence of infection within the herd (Raizman et al., 2004; Pillars et al., 2009) and also with the level of MAP shedding. The most contaminated areas on farms were manure storage areas and shared alleyways (Smith et al., 2011). MAP was also found elsewhere inside a barn, e.g. aerosols, dust, ventilation system, as well as in soil and water outside the barn (Raizman et al., 2004; Pillars et al., 2009). The latter may have a significant role in within-herd MAP infections (Singh et al., 2013). MAP in the environment of barns and pastures can serve as a source for infections of wildlife, which can spread the disease to other herds (Corn et al., 2005; Nugent et al., 2011).

MAP is a highly resistant bacteria and can survive in the environment for more than a year (Waddell et al., 2016a). In shade, protected from ultraviolet light and in a dry environment it survives for up to 55 weeks. Its survival is prolonged in water and sediment, while it is shortened in dry soil, with sunlight, changes in temperature, low pH, high ammonium and low iron level (Whittington et al., 2004;

Whittington et al., 2005; Eppleston et al., 2014). The success of the efforts to eliminate the bacteria from the environment is limited. After depopulation, mechanical and chemical disinfection a significant amount of MAP can still be present in the environment (Moravkova et al., 2012). Some researchers suggested that the bacteria may have a dormant phase, because some of the samples were unculturable during the study they conducted (Whittington et al., 2004; Whittington et al., 2005). Additionally, MAP can form biofilms, in which after one year the quantity of bacteria remains almost the same (Cook et al., 2010). MAP was also found in drinking water and was cultured from biofilms on the nets of taps (Pickup et al., 2005; Pickup et al., 2006; Pickup et al., 2009).

4.1.2 Potential risk

There are many studies connecting MAP with chronic diseases in humans. Even though there is still not enough evidence to confirm causality between MAP and human diseases, many researchers believe that there are reasons to suspect that it poses a risk for human health (Kuenstner et al., 2017). Likewise, some researchers caution against making such assumptions despite the lack of supporting evidence (Robertson et al., 2017).

MAP is assumed to be an infectious agent of Crohn's disease. One of the reasons for this assumption is that the clinical signs and affected tissues in Crohn's disease in humans are very similar to paratuberculosis in cattle (Crohn et al., 1932). Secondly, MAP was isolated from patients with Crohn's disease and significant associations were shown (Chiodini et al., 1984; Sanderson et al., 1992; Feller et al., 2007; Naser et al., 2014; Waddell et al., 2015). Additionally, treatment with antibacterial drugs was effective in some patients with Crohn's disease (Borody et al., 2007). However, the lack of correlation between Crohn's disease and isolation of MAP (Martin et al., 2008; Parrish et al., 2009; Mendoza et al., 2010; Van Kruiningen, 2011; Chiodini et al., 2012) and no effect in randomised clinical trials with antimicrobial drugs were also reported (Selby et al., 2007). Additionally, researchers caution that there is no difference between the occurrence of Crohn's disease in urban and rural areas. Furthermore, it was described as a disease that affects the affluent people (Economou & Pappas, 2007). Studies examining risk factors for Crohn's disease and/or exposure to MAP found an association with the consumption of cheese and processed meat, while other factors did not exhibit significant association. These factors were milk consumption, direct contact with ruminants, high risk occupations (farmer, veterinarian) and water source (Waddell et al., 2016b). The third reason why many believe MAP may be a zoonosis is because it can infect many domestic and wild animal species including non-human primates (Kuenstner et al., 2017). Some authors suggest that only some subpopulations may perhaps be susceptible; e.g. those with lower immune response and genetically susceptible individuals (Naser et al., 2004).

In addition to Crohn's disease, there are suggested links between MAP and irritable bowel syndrome, Blau syndrome, type 1 diabetes mellitus, Hashimoto thyroiditis, multiple sclerosis, Parkinson's disease and autism (Grant, 2015; EFSA, 2017). Some hypothesize that MAP is not a causative but rather an opportunistic agent in these diseases and that they merely enable the survival of MAP in the gastrointestinal tract (EFSA, 2017).

Even though details about the nature and consequences of the infection are unknown, it is important to improve the understanding of the risk of human exposure. The assumed most important source of

human exposure to MAP are infected cattle and their food products (Mihajlovic et al., 2011). The three most likely transmissions are believed to be consumption of contaminated milk and contaminated meat or organ tissues and drinking water contaminated with faeces from infected herds. Milk can be contaminated directly by cows shedding MAP in milk or indirectly. Indirect contamination is a consequence of faecal contamination and dirt from the udder, milking machines or other surfaces. This is possible even when good hygienic standards are enforced (Gill et al., 2011). Meat and other tissues can be contaminated with the dissemination of MAP in systemic infection as well as with external contamination of carcasses with faeces (Eltholth et al., 2009). Faeces from infected cattle may also contribute to the contamination of vegetables grown on contaminated soil and to environmental exposure to MAP (Gill et al., 2011).

Viable MAP was found in raw milk (Corti and Stephan, 2002; Stabel and Lambertz, 2004; Herthnek et al., 2008), beef (Savi et al., 2015) and drinking water (Pickup et al., 2005, 2006), as well as in processed food. MAP was shown to be able to survive many food processing steps, including pasteurisation of milk, cooking meat to a medium-rare and chlorine or chloramine treatment of water (Mihajlovic et al., 2011). For the pasteurisation two time/temperature regimes are set by WHO/FAO (2004): holder pasteurisation at 63°C for 30 minutes and high temperature short time (HTST) pasteurisation at 72°C for 15 seconds. At holder pasteurisation, inactivation of MAP was found to be non-linear and showed tailing due to the presence of MAP cells in clumps, which are more heat resistant than single cells. It was also observed that a longer exposure to heat is more effective than higher temperature and thus some MAP cells may stay intact after HTST pasteurisation when present in milk in high enough concentrations (ACMSF, 2000; Stabel and Lambertz, 2004; Grant et al., 2005). For UHT treatment of milk, it is believed that it deactivates MAP completely (ACMSF, 2000).

MAP was cultured from pasteurised milk (Millar et al., 1996; Grant et al., 2002a; Ellingson et al., 2005; Shankar et al., 2010; Carvalho 2012; Paolicchi 2012), retail cheese from pasteurised and raw milk (Collins, 1997; Ikonopoulou et al., 2005; Waddell et al., 2016b) and powdered infant formula (Botsaris et al., 2016). The level of contamination of different milk and milk products is highly variable between studies. However, in studies of pasteurised retail milk low levels of viable MAP were found in approximately 2% of samples (Grant et al., 2002b; Mihajlovic et al., 2011). On the other hand, there are also studies where MAP was not found in above-mentioned products (O'Doherty et al., 2002; Slana et al., 2009; Liandris, 2014). Nevertheless, in both cases, the problem with diagnostic tests should not be ignored. Even though milk and milk products are the most studied source of human exposure to MAP, it is not known what proportion of exposure to MAP they represent (Boulais et al., 2011).

4.2 EXPOSURE ASSESSMENT

4.2.1 Compartmental model

4.2.1.1 Distribution of cows in different health states

To demonstrate the realization of compartmental model simulations, one iteration of stochastic simulation is shown in Figure 6. As can be noted from this simulation, the number of cows per herd was changing over time. 1,000 iterations of the model through the whole study period resulted in an average of 17.49 cows per herd with the minimum 10 and maximum of 28 cows per herd. However, both extreme values were outliers and the 5th percentile (PC) was 17 and the 95th PC was 19 cows per herd. The variation in the herd size was the reason for expressing the results primarily in shares rather than absolute values.

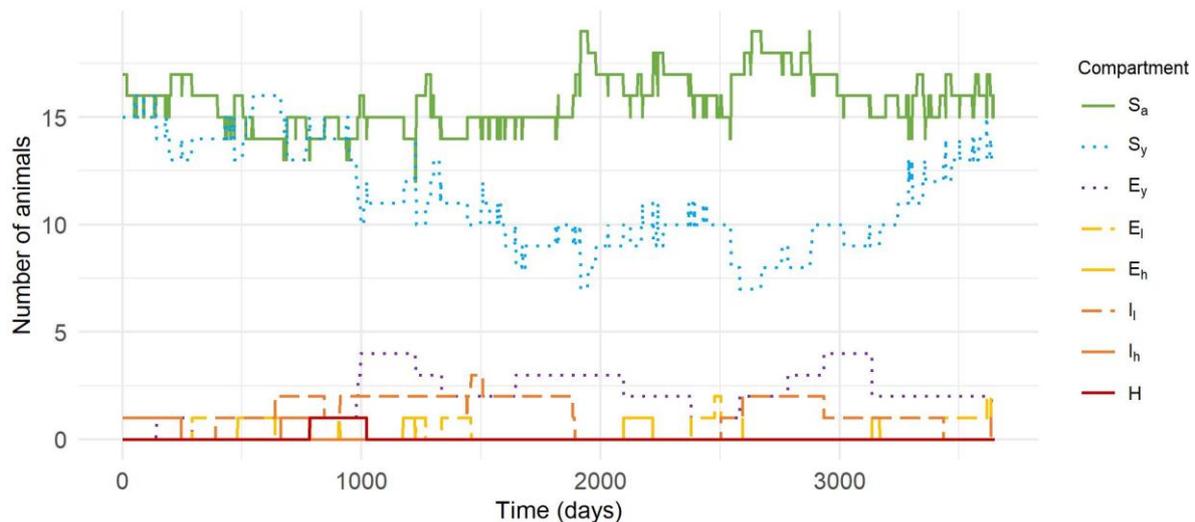


Figure 6: Demonstration of one iteration of the compartmental model simulations of the within-herd spread of MAP. Susceptible cows (S_a); susceptible young animals (S_y); latent young animals (E_y); latent cows on low shedding path (E_i); latent cows on high shedding path (E_h); infected cows on low shedding path (I_i); infected cows on high shedding path (I_h) and high shedding cows (H).

In Figure 7, we show the proportion of cows in each health state per herd through the entire simulation period. For all MAP positive health states, the median value was zero, whereas the median for susceptible cows was 94.44% or approximately 16 cows. In no case was the herd fully infected. The minimum proportion of susceptible cows was 29.41% and the maximum was 100%, meaning that paratuberculosis was able to fade out. There were on average 92.87% susceptible cows per herd with standard deviation (SD) of 8.42%. The mean values for other health states were lower than one percent for a latently infected cow on the high shedding path as well as for high shedding or clinical cows, 0.83% (2.28% SD) and 0.47% (1.63% SD) respectively. There were on average 1.42% (2.74% SD) infected cows on the high shedding path, 1.83% (3.73% SD) of latently infected cows on the low shedding path and 2.58% (4.55% SD) of infected cows on the low shedding path per herd. For infected cows on both paths or subclinical cows, the mean value was 4% (5.42% SD) with a median of zero. The maximum of subclinically and clinically infected cows were 18.75% and 50% per herd, respectively. For MAP positive health states, the 95th PC was one cow per herd in the high shedding path and two cows on the low

shedding path. All these compartments combined gave the mean within-herd prevalence of 7.13% (8.43% SD) with the 95th PC being 23.53% and maximum prevalence of 70.59%. The frequency distributions of the prevalence, subclinically infected and clinically infected cows are presented in Figure 8.

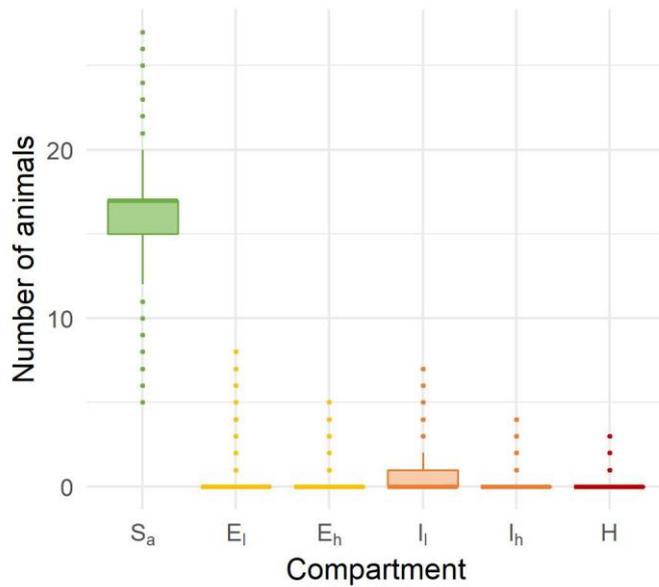


Figure 7: Distribution of cows in each health state in the compartmental model of the within-herd spread of MAP. Susceptible cows (S_a); latent cows on low shedding path (E_l); latent cows on high shedding path (E_h); infected cows on low shedding path (I_l); infected cows on high shedding path (I_h) and high shedding cows (H).

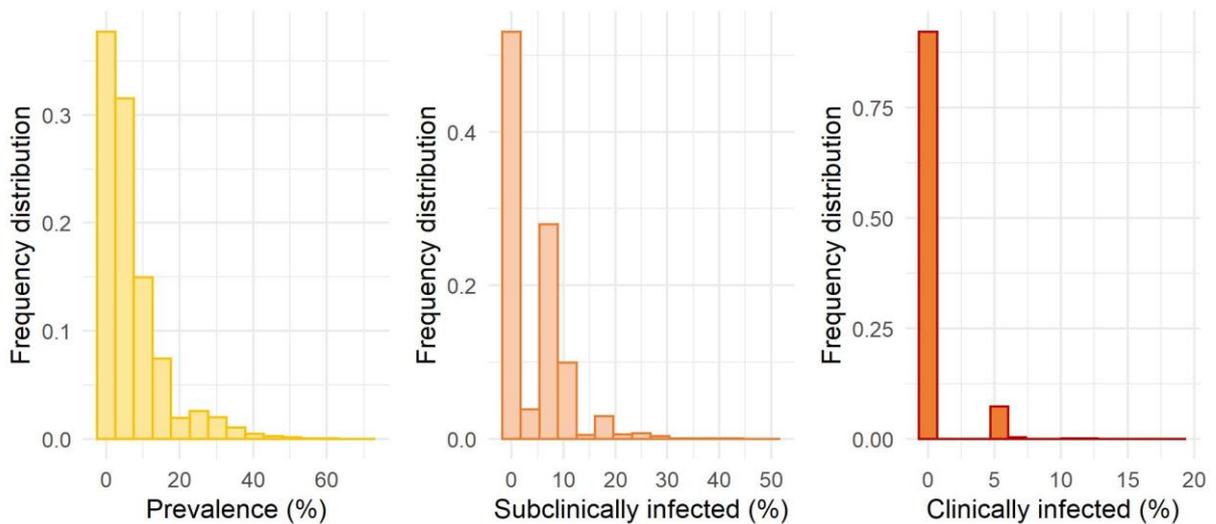


Figure 8: Frequency distribution of the within-herd prevalence of MAP and proportion of subclinically and clinically infected cows.

Correlations between the proportion of clinically and subclinically infected cows and prevalence were all highly statistically significant (adjusted p-values < 0.0001) (Figures 9 and 10). The Spearman's rank correlation coefficient showed a strong correlation between the proportion of subclinical cows and prevalence (Spearman's $\rho = 0.79$), while the correlation between the proportion of clinical cows and prevalence was weak (Spearman's $\rho = 0.31$). Interestingly, the correlation between the proportion of clinical cows and subclinical cows was very weak (Spearman's $\rho = 0.04$).

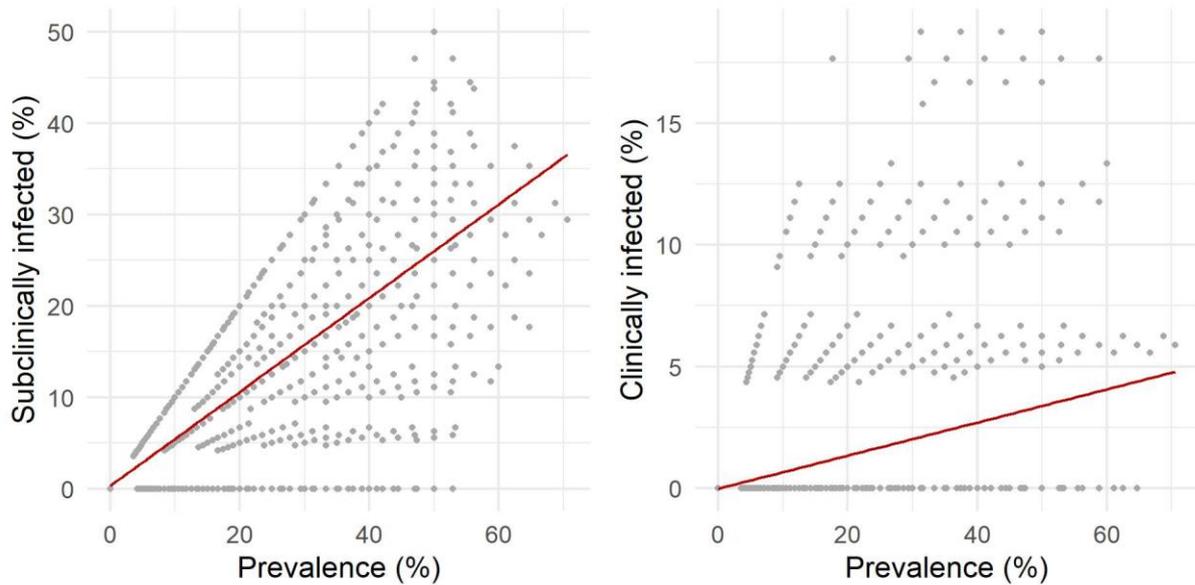


Figure 9: Correlations between MAP within-herd prevalence and proportion of subclinically and clinically infected cows per herd.

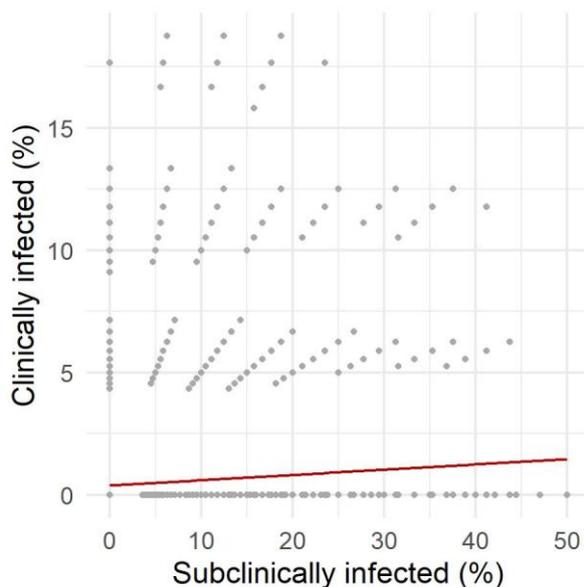


Figure 10: Correlation between the proportion of subclinically and clinically infected cows in a MAP positive herd.

4.2.1.2 Sensitivity analysis

In the sensitivity analysis we examined the effect of change in eight epidemiologically most important parameters on the results of within-herd prevalence of MAP and proportion of subclinically and clinically infected cows per herd (Tables 7, 8 and 9). Overall, the results were in line with our expectations. The parameters with their minimum and maximum values that had the greatest impact on the simulation results were the transmission rate from infected cows to young animals (β_{yi}) and the transmission rate of infected cows (β_i). The minimum values of these two parameters resulted in lower numbers of infected cows, whereas the maximum values more than doubled the within-herd prevalence. Other parameters with noticeable effects were: the animal-level prevalence (P), the transmission rate from high shedding cows to young animals (β_{yh}) and the transmission rate for high shedding cows (β_h). The transition rate from a latent to an infected cow (σ), the progression from an infected cow on the high shedding path to a high shedding cow (ω) and the proportion of latently infected young animals entering the high shedding path (α) had only minor influence on the results.

Table 7: Results of MAP within-herd prevalence from the sensitivity analysis of epidemiologically most important parameters.

Parameter	Min/max value	5 th PC	1 st quantile	Median	Mean	SD	3 rd quantile	95 th PC
Basic model	/	0	0	5.56	7.13	8.43	11.11	23.53
σ	min	0	0	5.56	6.24	7.31	10.53	22.22
σ	max	0	0	5.56	6.53	7.63	11.11	22.22
ω	min	0	0	5.56	5.38	6.28	5.88	17.65
ω	max	0	0	5.56	6.63	7.85	11.11	23.53
β_i	min	0	0	5.56	6.83	8.37	11.11	23.53
β_i	max	0	5.26	11.76	16.88	17.32	27.78	52.94
β_h	min	0	0	5.56	6.49	7.47	11.11	22.22
β_h	max	0	0	5.88	12.46	15.43	17.65	47.06
P	min	0	0	0	4.08	6.89	5.88	17.65
P	max	0	5.56	11.11	12.73	11.3	17.65	35.29
β_{yi}	min	0	0	5.56	5.6	6.98	5.88	17.65
β_{yi}	max	0	5.88	16.67	20.22	17.88	31.58	55.56
β_{yh}	min	0	0	5.56	5.26	6.38	5.88	17.65
β_{yh}	max	0	0	5.88	12.09	13.85	17.65	41.18
α	min	0	0	5.88	7.32	8.22	11.76	23.53
α	max	0	0	5.88	7.54	8.93	11.76	26.32

Table 8: Proportion of subclinically infected cows from the sensitivity analysis of epidemiologically most important parameters.

Parameter	Min/max value	5 th PC	1 st quantile	Median	Mean	SD	3 rd quantile	95 th PC
Basic model	/	0	0	0	4	5.42	5.88	15.79
σ	min	0	0	0	3.05	4.41	5.88	11.76
σ	max	0	0	0	3.46	4.8	5.88	11.76
ω	min	0	0	0	3.82	4.91	5.88	11.76
ω	max	0	0	0	4.17	5.47	5.88	15.79
β_i	min	0	0	0	3.98	5.46	5.88	15.79
β_i	max	0	0	5.88	9.34	10.84	14.29	31.58
β_h	min	0	0	0	3.76	4.99	5.88	11.76
β_h	max	0	0	0	3.76	4.99	5.88	11.76
P	min	0	0	0	2.32	4.33	5.56	11.76
P	max	0	0	5.88	7.41	7.49	11.76	23.53
β_{yi}	min	0	0	0	3.4	4.75	5.88	11.76
β_{yi}	max	0	0	5.88	10.55	10.83	17.65	33.33
β_{yh}	min	0	0	0	3.19	4.5	5.88	11.76
β_{yh}	max	0	0	5.56	6.68	8.36	11.11	23.53
α	min	0	0	4.76	4.39	5.63	5.88	16.67
α	max	0	0	0	4.09	5.5	5.88	16.67

Table 9: Proportion of clinically infected cows from the sensitivity analysis of epidemiologically most important parameters.

Parameter	Min/max value	5 th PC	1 st quantile	Median	Mean	SD	3 rd quantile	95 th PC
Basic model	/	0	0	0	0.47	1.63	0	5.56
σ	min	0	0	0	0.25	1.17	0	0
σ	max	0	0	0	0.33	1.34	0	5.56
ω	min	0	0	0	0	0	0	0
ω	max	0	0	0	0.24	1.19	0	0
β_i	min	0	0	0	0.45	1.61	0	5.56
β_i	max	0	0	0	0.54	1.73	0	5.88
β_h	min	0	0	0	0.42	1.54	0	5.56
β_h	max	0	0	0	0.42	1.54	0	5.56
P	min	0	0	0	0.27	1.23	0	0
P	max	0	0	0	0.71	2.02	0	5.88
β_{yi}	min	0	0	0	0.4	1.49	0	5.56
β_{yi}	max	0	0	0	0.88	2.28	0	5.88
β_{yh}	min	0	0	0	0.39	1.49	0	5.56
β_{yh}	max	0	0	0	0.65	1.96	0	5.88
α	min	0	0	0	0.36	1.42	0	5.56
α	max	0	0	0	0.57	1.8	0	5.88

4.2.2 Network analysis

4.2.2.1 Description of cattle movements

Out of 2,277,166 recorded individual animal movements from 1st August 2011 to 31st July 2016, 1,020,860 met the inclusion criteria (Table 10). Movements within Slovenia represented 71.3%, the rest were animals imported and exported from or to EU and non-EU countries. For imported animals, only the ones intended for further breeding were accounted for, whereas for the export, animals moved to end nodes were also included. Within the country, 543,180 animals were moved from one to 28 times, the majority of them (97.4%) being moved up to three times. Collectively, 37,138 premises were involved in cattle trade; namely: 36,885 farm holdings, 36 pastures, 174 mountain pastures, 16 exhibitions, 3 fairs and 24 collection centres. Not all premises were active during the entire study period, several have ceased activities and few premises were newly established. This is also evident from the national statistical data since the number of farm holdings with cattle was decreasing through time. In 2016, there were 32,805 farm holdings with cattle registered in Slovenia, which was 3,314 less than in 2010⁵.

Table 10: Summary of cattle movement data included in the study.

Study year	Year 1	Year 2	Year 3	Year 4	Year 5	Total
Study period	Aug 2011– Jul 2012	Aug 2012– Jul 2013	Aug 2013– Jul 2014	Aug 2014– Jul 2015	Aug 2015– Jul 2016	Aug 2011– Jul 2016
No. of active premises	29,172	29,004	28,395	28,195	28,008	37,138
No. of animal movements	142,142	144,589	144,050	144,834	151,992	727,607
No. of imported cattle for further breeding	20,510	18,650	20,281	23,673	25,419	108,533
No. of exported cattle	38,073	40,970	39,184	30,748	35,745	184,720

More than half of all movements in Slovenia created direct farm-to-farm contacts and almost a third (29.3%) went through collection centres (Figure 11). The number of movements by month revealed a strong seasonal pattern, each year with the maximum number of movements in September and another peak in June. The minimum number of movements was in February, except for the last studied year when January was the least active month (Figure 12). The number of imported cattle intended for further breeding by month also showed consistency throughout all five years with peak activity in autumn. Most of these animals were imported from the Czech Republic, Hungary, Romania, Slovakia and Austria (58.2%, 10.2%, 9.5%, 9.2% and 3.4%, respectively). Overall, approximately half of the animals were imported directly to farm holdings and half went through collection centres. The proportion of animals going through collection centres increased every year; from 41% in the first year to 61% in the last studied year. The export movements did not show pronounced consistency or seasonality. In the last studied year, Slovenia exported 35,745 animals. Overall, most animals (88.9%) were exported to Austria, Italy and Lebanon.

⁵ https://pxweb.stat.si/SiStatDb/pxweb/en/20_Ekonomsko/20_Ekonomsko__24_zunanja_trgovina__01_lzvoz_in_uvoz_blaga__02_Podatki_KN__8-mestni_KN/

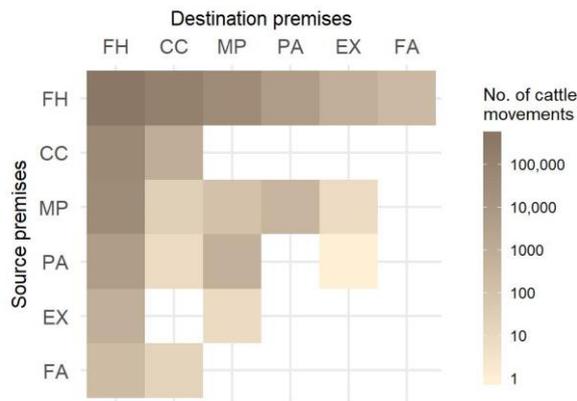


Figure 11: Number of animal movements between different types of premises from 1st August 2011 to 31st July 2016. Legend: FH – farm holdings; CC – collection centres; MP – mountain pastures; PA – pastures; EX – exhibitions; FA – fairs.

4.2.2.2 Characterization of the cattle trade network

Table 11: Summary of global measures of yearly and monthly cattle movement networks. The given numbers are mean values over time.

	Yearly networks		Monthly networks	
	Mean [range]		Mean [range]	
No. of active premises	28,555	[28,008–29,172]	7,926	[6,782–9,128]
No. of movements	145,521	[142,142–151,992]	12,127	[8,364–18,415]
No. of batches	60,120	[59,310–61,262]	6,665	[5,448–7,817]
Density	4.36×10^{-05}	$[4.30 \times 10^{-05} - 4.44 \times 10^{-05}]$	4.83×10^{-06}	$[3.95 \times 10^{-06} - 5.67 \times 10^{-06}]$
Avg. degree	3.24	[3.19–3.30]	0.36	[0.29–0.42]
Avg. shortest path length	9.34	[8.85–10.16]	2.71	[1.72–5.52]
Diameter	28.60	[25–37]	9.25	[7–16]
Degree assortativity	-0.0645	[-0.0653 to -0.063]	-0.0689	[-0.157 to -0.047]
Clustering coefficient	0.0017	[0.0016–0.0018]	7.47×10^{-04}	$[4.41 \times 10^{-04} - 1.43 \times 10^{-03}]$
Size of GWCC	27,828	[27,331–28,416]	3,645	[2627–4960]
% of active holdings	97.46	[97.15–97.82]	45.82	[36.88–56.48]
Size of GSCC	5,185.60	[4,791–5,455]	12.92	[3–73]
% of active holdings	18.17	[16.42–19.48]	0.16	[0.04–0.93]

Legend: GWCC – giant weakly connected component; GSCC – giant strongly connected component.

Global network measures for monthly and yearly networks are summarized in Table 11. The total number of active nodes in yearly networks decreased through the years. Based on node types we saw that only the number of active farm holdings dropped each year, from 78.5% to 75.3% in the last year, whereas the number of active nodes per other types did not change considerably. Each year, there were 20 active collection centres. There were one to a maximum of two fairs per year and two to seven exhibitions. The number of active pastures and mountain pastures varied mildly; in the first year, there were 26 active pastures and 32 in the last year, while the number of active mountain pastures was 170 in the first year and 162 in the last year. The minimum number of arcs was noted in the first year and the maximum number in the last studied year. This does not apply for the number of batches since the

minimum number of batches was recorded in the last year. In monthly networks, the number of active farm holdings, pastures and mountain pastures showed seasonal patterns, whereas the number of active collection centres, fairs and exhibitions varied very little between months. The number of arcs varied considerably between months, but very little in the same month in different years.

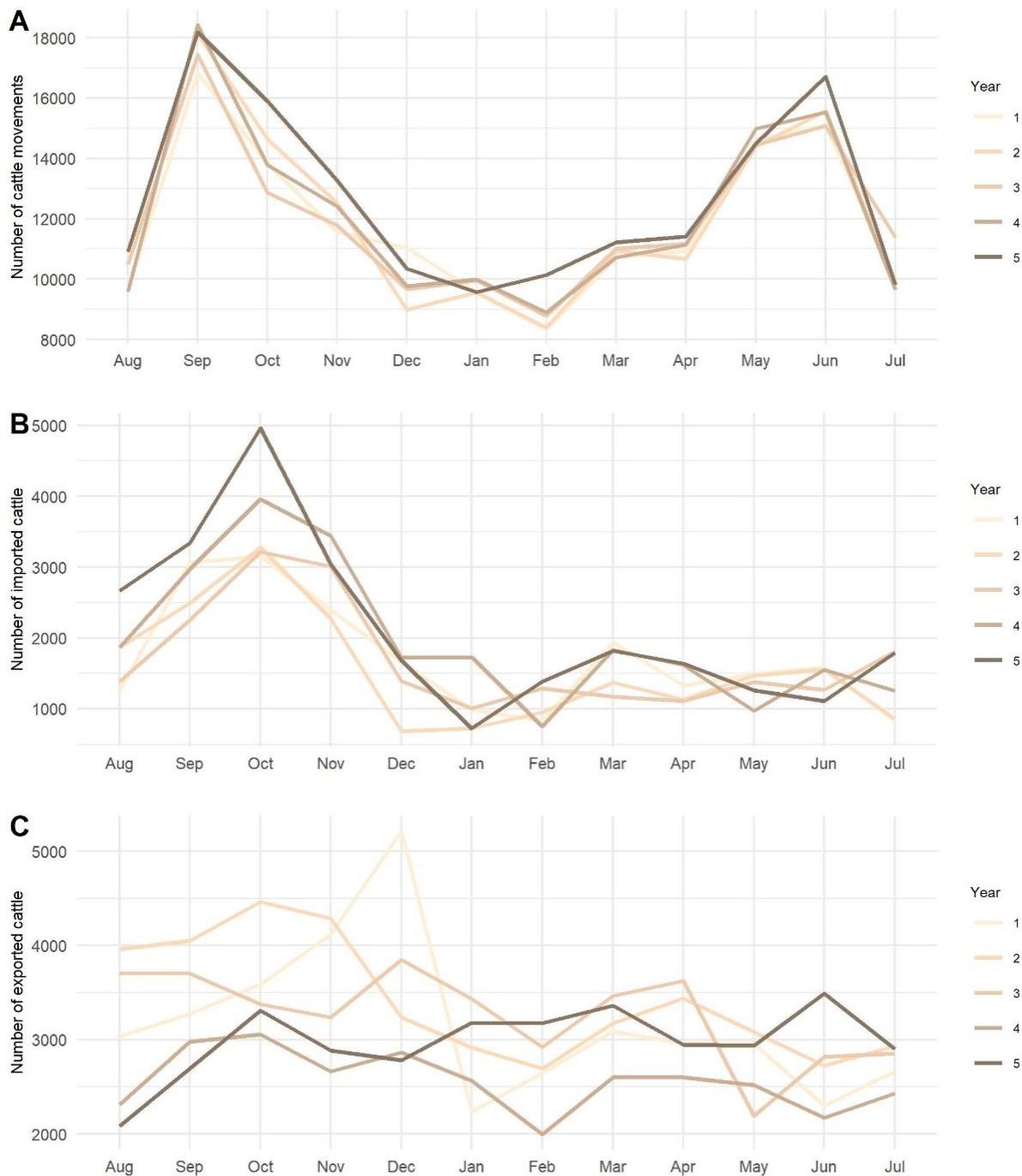


Figure 12: Number of animal movements by month from 1st August 2011 to 31st July 2016. (A) Number of cattle movements in Slovenia without movements to end nodes, (B) number of cattle imported for further breeding and (C) number of exported cattle including movements to end nodes.

The arc density shows that both yearly and monthly networks are sparse since on average only $4.36 \times 10^{-3}\%$ and $4.83 \times 10^{-4}\%$ of contacts are realized, respectively. In the yearly networks, the total degree of a single node ranged between zero and 1,757, with the mode being one and median two. The total degree in a monthly network ranged between zero and 364, with both the mode and median being zero. The average shortest path length showed that on average, approximately nine animal movements are connecting a pair of randomly chosen active premises in the yearly networks and three animal movements in monthly networks. The average network diameter or the longest shortest path between two active nodes in a network required approximately 29 steps in the yearly and nine in the monthly networks. All networks had negative degree assortativity, which means that a connection between nodes with different degrees is more likely than a connection between nodes with a similar degree. The small values of the clustering coefficients showed that the clustering in the analysed networks was weak. A Mann-Kendall test did not show any trend in the monthly network measures.

In each of the studied yearly networks, almost all active nodes were part of GWCC, whereas less than half of all active nodes in the monthly networks were also part of the GWCC. The yearly GWCC on average included 75% of all nodes (range: 73.6–76.5%). In monthly networks, the proportion of nodes in the GWCC was lower, on average 9.81% and ranged between 7.1% and 13.4%. The sizes of the second largest weakly connected components were significantly smaller and included from 6 to 9 nodes in yearly networks and from 17 to 120 nodes in monthly networks. In the yearly networks, the GSCCs contained on average 14% of all nodes, with a minimum number of nodes in the first year and a maximum in the last studied year. The second largest strongly connected component included from seven to 21 nodes with the average being 13.4 nodes. There were one to four strongly connected components with more than ten nodes per year. The GSCCs in each monthly network contained less than one percent of active nodes. GSCCs with more than 20 nodes were observed in June, July, August and October. The second largest strongly connected component on average consisted of six nodes (range: 3–16 nodes). In the large majority of months, only one strongly connected component included more than ten nodes.

From the epidemiological perspective, the component structure is an important feature of networks since it determines possible paths for the transmission of disease and the maximum size of disease outbreaks. A so-called bow-tie partition (Figure 13A) assigns nodes into six different components; namely GSCC, *giant in component* (GIC), *giant out component* (GOC), *tubes*, *tendrils* and *others* (Broder et al., 2000). The size of the GWCC equals all the nodes in the network minus nodes in the component *others*. The situation is most unfavourable if an outbreak starts in nodes that belong to the GSCC or GIC because there are many available paths for the spread of disease. On the contrary, a disease originating in nodes from other components can reach only a limited number of nodes (Lentz et al., 2016). Figure 13 displays the structure of the bow-tie partition of the average yearly network in the studied period and the mean percentage of nodes belonging to each component. The affiliation of nodes to different bow-tie components is not constant over the years. In fact, more than half of the nodes changed their affiliation each year, which shows that even though the node level measures were highly correlated, the overall connectedness of the premises to other parts of the network tended to change over time (Figures 13B and 14).

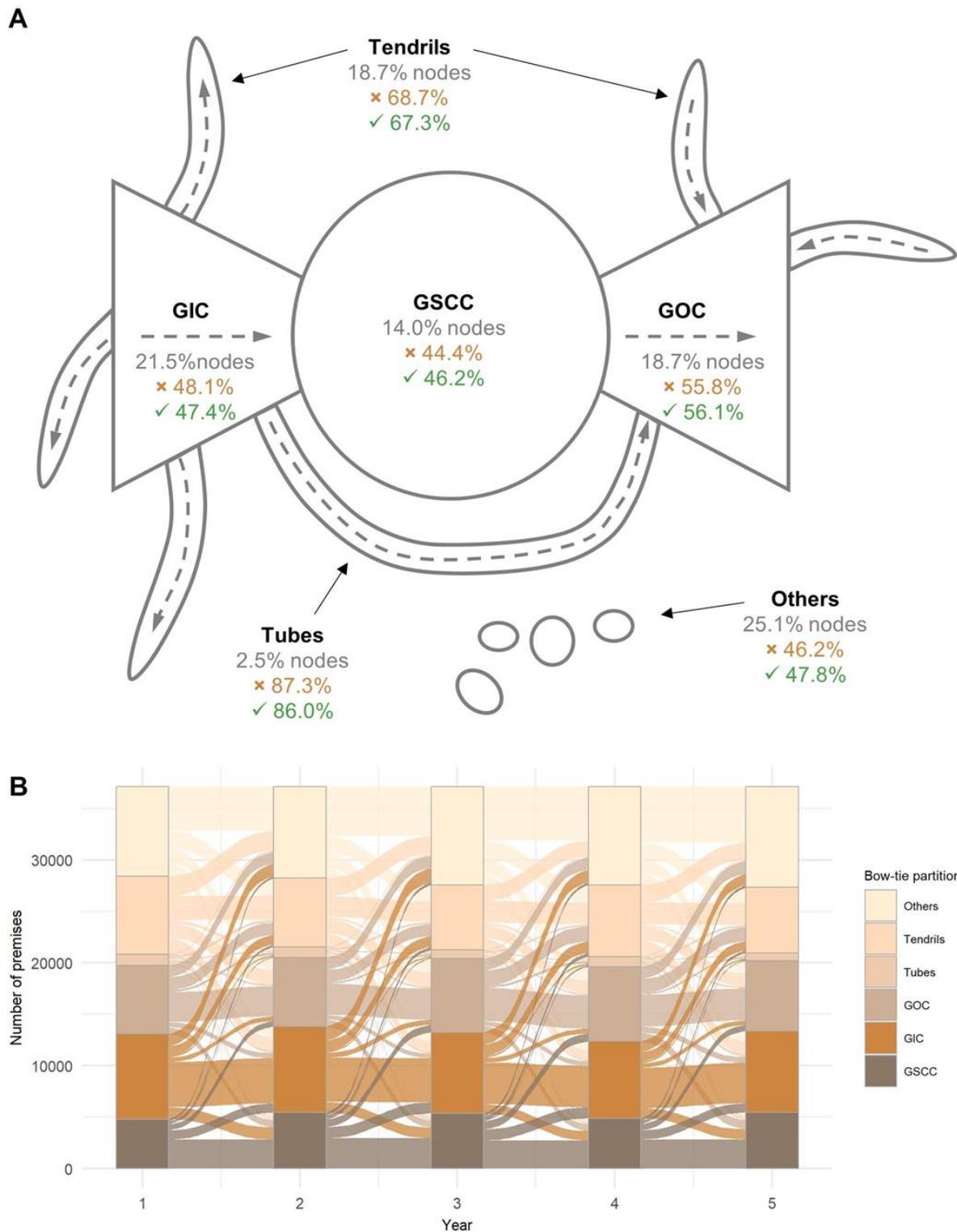


Figure 13: Bow-tie partition. (A) Structure of the bow-tie partition of the yearly cattle movement networks. Grey numbers are the average percentage of nodes in a component. The percentage of nodes exiting the component is denoted with (×) and entering the component is denoted with (✓) (Broder et al., 2000). (B) Transition plot showing the sizes of components per year and the transition between bow-tie components. Legend: GSCC – giant strongly connected component; GIC – giant in component; GOC – giant out component.

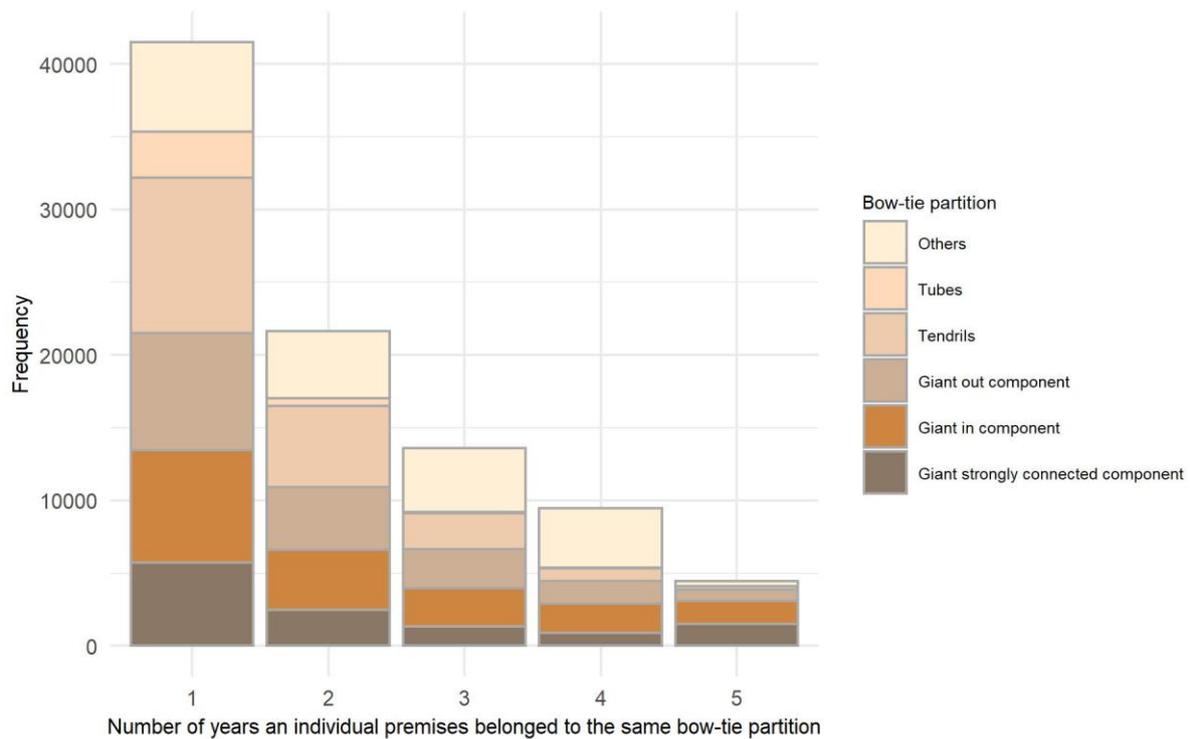


Figure 14: Frequency of an individual premises belonging to the same bow-tie partition in one year or repeatedly in multiple years.

Local network measures showed considerable consistency across the studied period. Correlations of all measures between years were moderate to strong and highly statistically significant (adjusted p-values < 0.0001). For all measures, the minimum Spearman’s rank correlation coefficients were found when comparing first and last studied year (Spearman’s $\rho = 0.39\text{--}0.61$), whereas all except one maximum coefficient were found between the fourth and fifth year (Spearman’s $\rho = 0.52\text{--}0.72$). An exception was the correlation coefficient for out-closeness centrality, for which the maximum value was found between the third and fourth year.

Correlations between local network measures were also highly statistically significant (adjusted p-values < 0.0001). Degree, weighted degree, betweenness and closeness centralities were moderate to very strongly correlated with all other network measures (Spearman’s $\rho = 0.44\text{--}0.94$). Centralities based on in-going movements in-degree, in-weighted degree and in-closeness centrality were very strongly correlated between each other (Spearman’s $\rho = 0.91\text{--}0.97$). The same applies to centralities concerning out-going movements. A very weak correlation was found only when comparing in-going and out-going centralities (Spearman’s $\rho = 0.08\text{--}0.16$).

Figure 15 shows cumulative distributions of the total, in- and out-degree in yearly and monthly networks on a logarithmic scale. Distributions were heavy-tailed with more or less apparent bimodal structure, which is a consequence of a large proportion of nodes with relatively low degree values and a small number of nodes with extremely high values of degree centrality. In the yearly networks, ten percent of nodes with the highest total degree centrality accounted for approximately 54% of all movements, top 20% of nodes for 70% of movements and top 30% of nodes for 80% of movements. The variation between years was very low and the range was covered with less than a 1.2 percentage point difference.

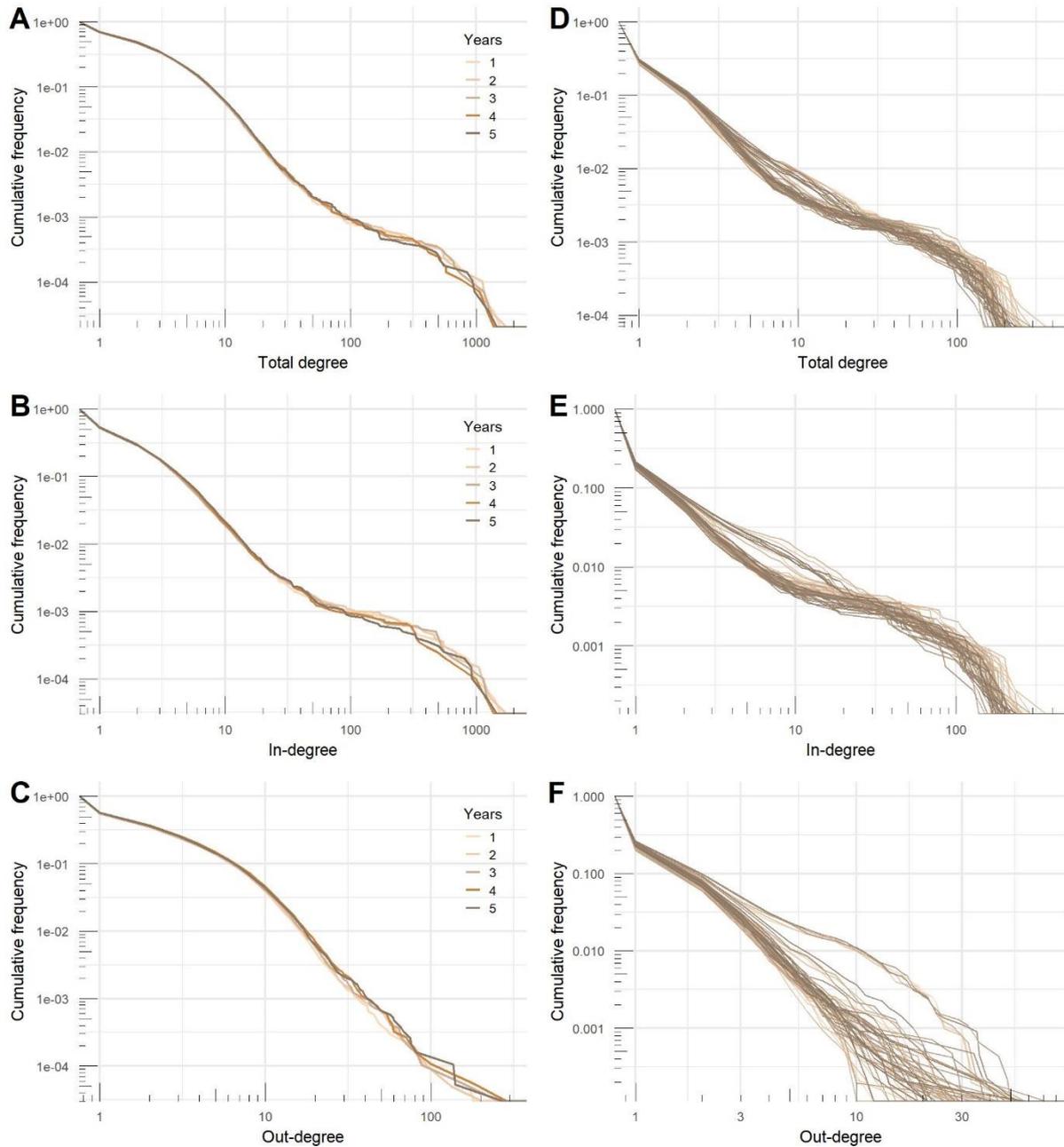


Figure 15: Degree distributions of (A) yearly total degree, (B) in-degree and (C) out-degree, and (D) monthly total degree, (E) in-degree and (F) out-degree of cattle movement networks.

For all node level measures, there were statistically significant differences between types of premises (Figure 16). Mountain pastures significantly differed from farm holdings and exhibitions in all measures as well as exhibitions from pastures. No significant difference was found between fairs in relation to farm holdings and exhibitions. Collection centres were shown to be the most influential type of premises in the global networks since they had the highest values of all observed measures.

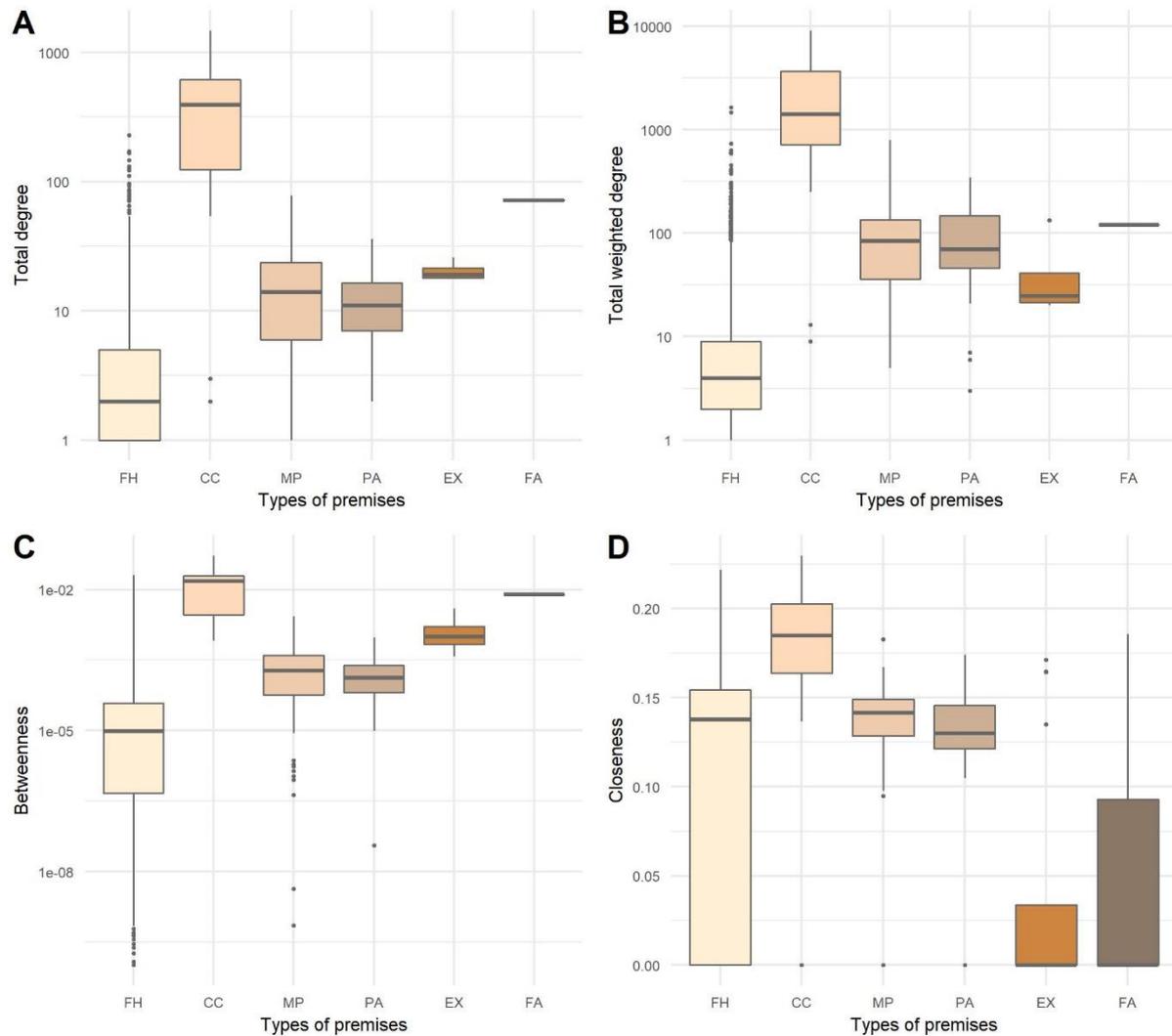


Figure 16: Distributions of (A) total degree, (B) total weighted degree, (C) betweenness and (D) closeness centralities by type of premises in the last year of the studied cattle movement network. Legend: FH – farm holdings; CC – collection centres; MP – mountain pastures; PA – pastures; EX – exhibitions; FA – fairs.

Compared to the cattle trade networks, the random networks with the same number of nodes and arcs exhibited less structuredness and more homogeneous connectedness. The average shortest path length and diameter were higher than observed in the trade networks. The clustering coefficient was one to two folds lower. At the same time, the giant components included many more nodes than our studied networks; GWCC 95% and GSCC 40% of all nodes.

As demonstrated in Figure 17, the most successful strategy to reduce the size of GSCC was node removal based on the total degree centrality in the fifth year, hence in the same year as the removal was performed. When we removed 0.1% of all nodes in the network, the results were quite similar for degree and betweenness centrality; the mean sizes of the remaining GSCCs were 59.7% and 60.3%, respectively, and the variation between years was low. Closeness centrality was less efficient and the mean size of remaining GSCC was 69.3% after removing 0.1% of nodes and 53.7% after removing 1% of all nodes. The difference between degree and betweenness centrality became apparent when we removed 0.5% of nodes, the sizes of remaining GSCCs ranged from 26.0% to 38.5% and 32.9% to 45.8%, respectively. It also became apparent that using the latest data gives better results. After removing 1% of nodes based on the values for the fourth year, the GSCC size dropped to 18.4% when using ordering based on betweenness and 6.5% when using the total degree.

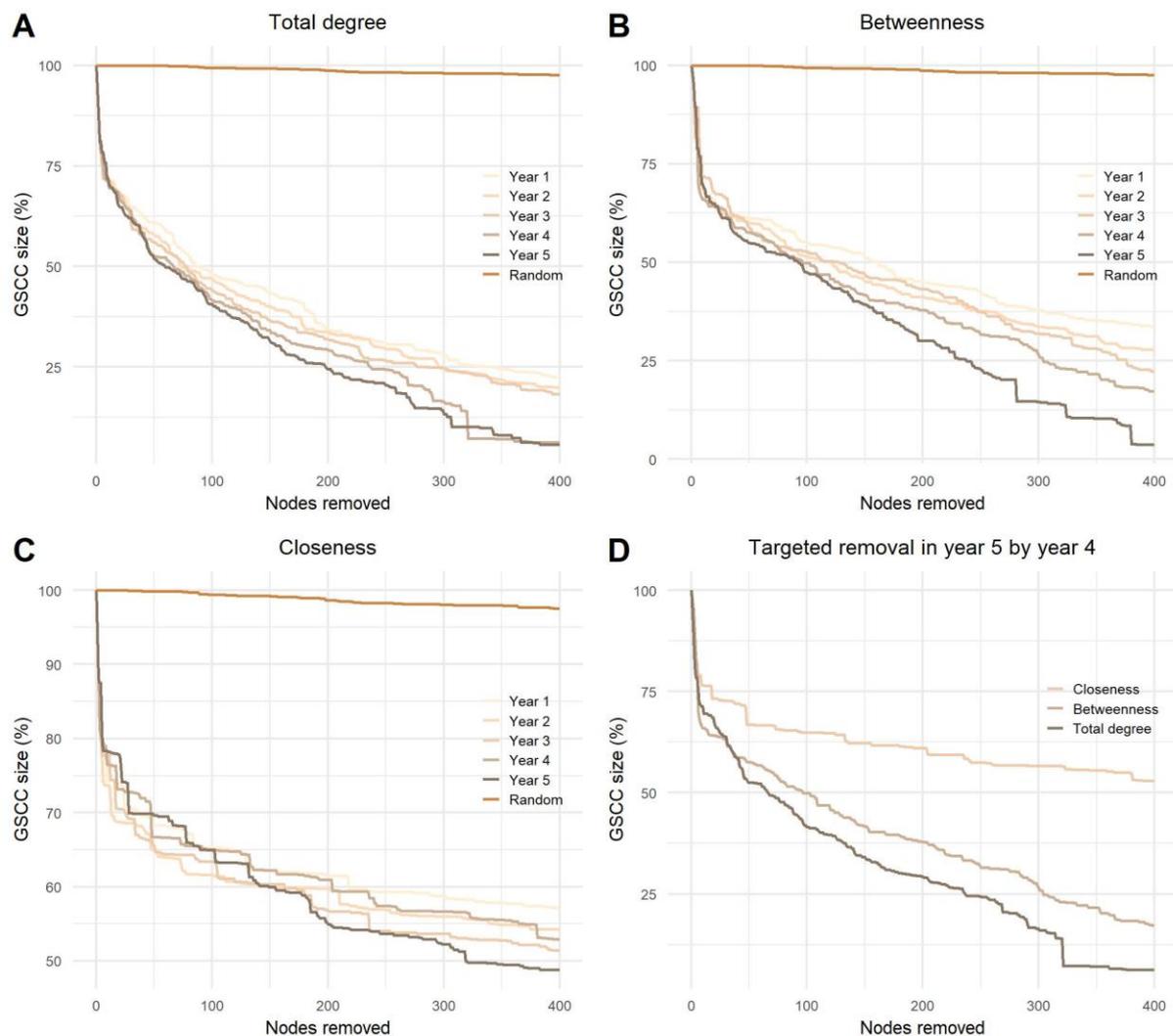


Figure 17: Targeted node removal in the fifth yearly network based on (A) total degree, (B) betweenness and (C) closeness centrality from the first to the last studied year and (D) comparison of effectiveness between these three centralities in the fourth yearly network on the disintegration of the GSCC in the fifth yearly network. Legend: GSCC – giant strongly connected component.

4.2.2.3 SI model of possible paratuberculosis spread on the temporal network

Figure 18 presents the results of the SI model on the temporal network predictions of possible MAP spread between cattle herds in Slovenia. The probability of transmission largely affected the proportion of farms being infected and the velocity of spread on the network. At the end of the study period, the predicted proportion of newly infected herds obtained with one thousand realizations for our chosen transmission probabilities of 0.02, 0.15, 0.25 and 0.40 were: 2.81%, 20.40%, 30.72% and 41.36%, respectively. As expected, the higher was the probability of transmission, the steeper were the slopes of infection curves. Even though we started the simulation with randomly selected initially infected farm holdings and one hundred different diluted networks, the variations in the proportion of infected herds for each transmission probability were low. Standard deviation was increasing with time and reached the maximum towards the end of the study period, except for the transmission probability of 0.40, where the maximum standard deviation was observed at day 1,073. Maximum standard deviations from the lowest to the highest probability of transmission were: 0.01%, 0.31%, 0.33% and 0.32%. The average numbers of newly infected farm holdings per day were: 0.57, 4.12, 6.20 and 8.34, respectively (Figure 19). Four distinct peaks in the incidence rate can be observed, concentrated mainly in September and October. In none of the scenarios, the infection reached a saturation; however, in the estimated and worst-case scenario, the velocity of disease spread decreased towards the end of the study period.

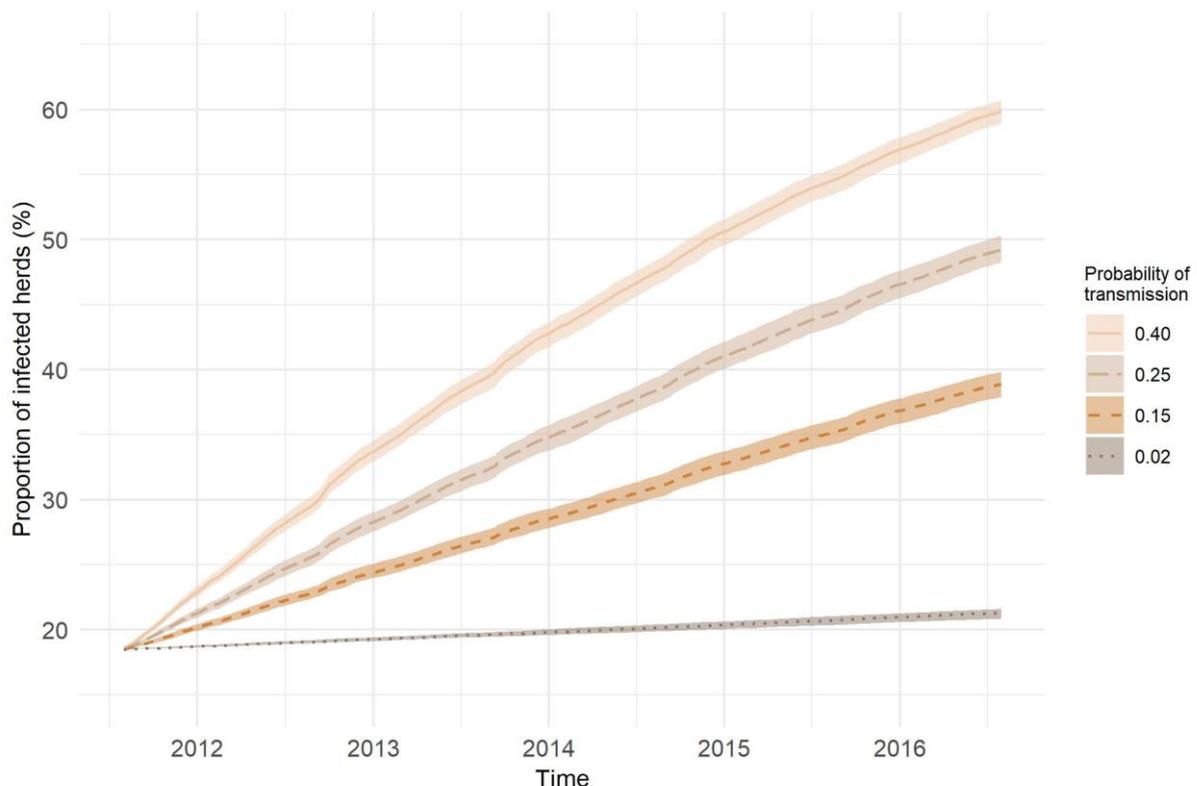


Figure 18: SI infection curves (lines – average over 1000 realizations; area – range) in the temporal network representation of cattle movements for different probabilities of transmission.

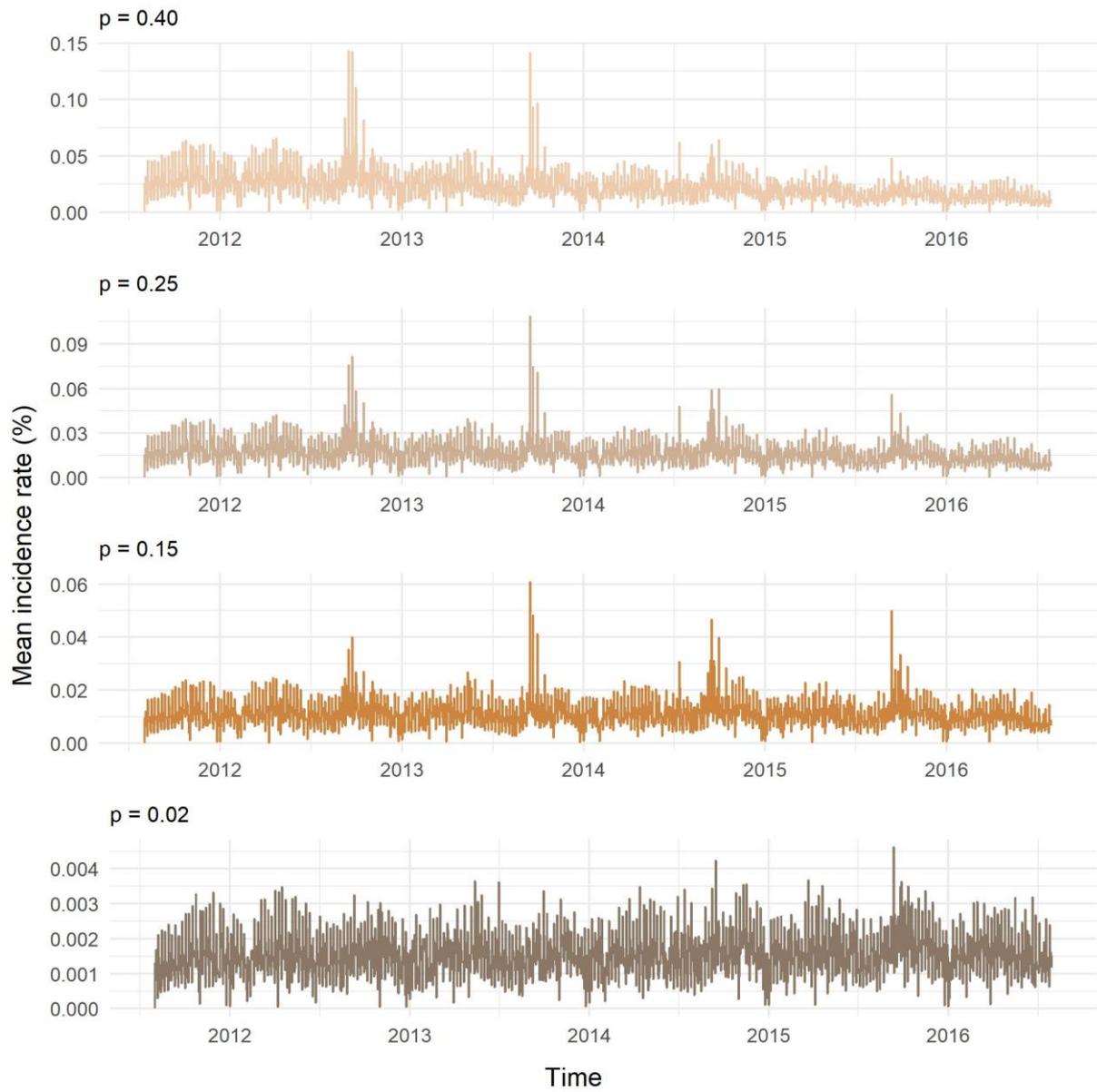


Figure 19: Mean daily incidence rate of newly infected farm holdings for different probabilities of transmission (p).

4.2.3 Human exposure assessment

4.2.3.1 MAP contamination of raw and pasteurised milk at a MAP positive farm

The concentration of MAP at a positive farm (MAP_{herd}) ranged between 0.01 to 5.49 log CFU per litre of raw bulk tank milk. The mean concentration per bulk tank milk was 0.46 log CFU per litre with 0.48 log CFU per litre SD. The concentration of MAP in a litre of raw milk ($MAP_{herd\ raw}$) was found to vary from zero to a maximum of almost 310 thousand MAP CFU per litre. On average, a litre of raw milk at a positive farm contained 164 MAP CFU with a 90% confidence interval (CI) of ± 77.58 MAP CFU and 5th and 95th PC being zero and 22 MAP CFU per litre, respectively. The mode was one and median two MAP CFU per litre of raw milk. The model estimated that 86.67% of litres of milk contained MAP cells, among which 1.83% of litres contained at least one hundred MAP cells. The probability distributions of raw milk contamination with MAP are shown in Figure 20.

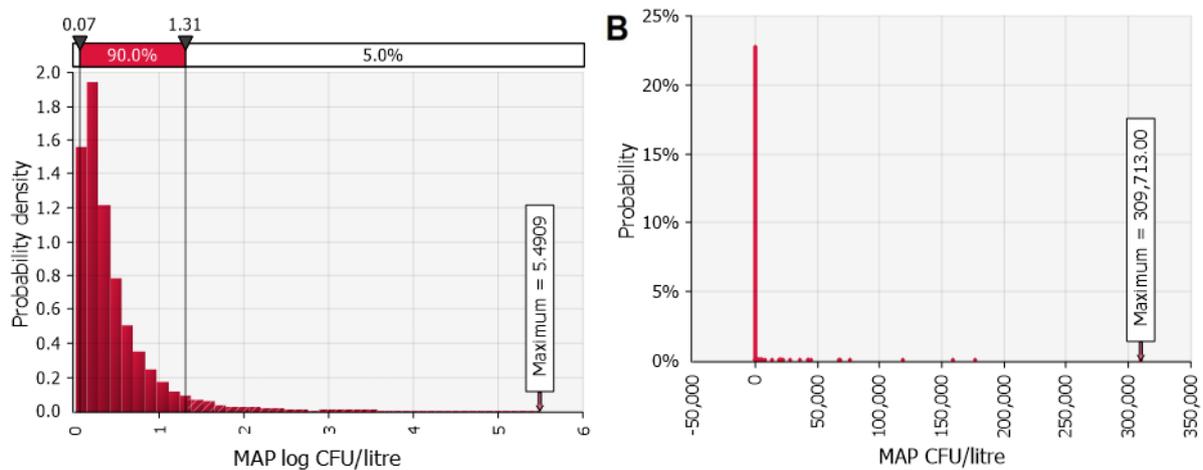


Figure 20: MAP contamination of raw milk at a MAP positive farm. (A) Probability density of MAP contamination of raw bulk tank milk. (B) Discrete probability of MAP contamination per litre of raw milk.

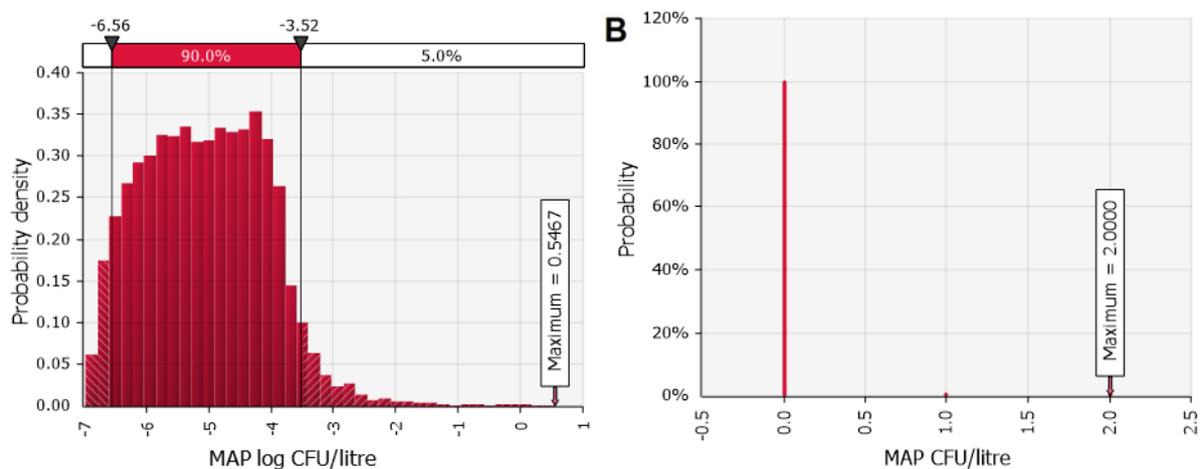


Figure 21: MAP contamination of pasteurised milk at a MAP positive farm. (A) Probability density of MAP contamination of pasteurised milk at the farm level. (B) Discrete probability of MAP contamination per litre of pasteurised milk.

After pasteurisation at the farm ($MAP_{herd\ past}$) the estimated proportion of litres of milk containing MAP cells was much lower, only 0.08% litres of milk contained MAP cells. Figure 21 shows the probability distributions for MAP contamination in pasteurised milk at the farm level. The mean concentration of MAP in pasteurised milk at the farm level was -5.33 log CFU per litre with the 90% CI of ± 0.02 log CFU per litre and ranged between -6.95 to 0.55 log CFU per litre. The estimated maximum contamination of a litre of pasteurised milk at a MAP positive farm was 2 MAP cells. The mean contamination was 0.001 and the 99th PC was zero MAP cells per litre of milk.

4.2.3.2 MAP contamination of pasteurised and UHT milk at the industry level

At the industry level, we simulated the collection of raw milk from one hundred dairy farms with three scenarios of paratuberculosis herd level prevalence. These were scenario 1, 2 and 3 with mean paratuberculosis prevalence of 21.3%, 38.89% and 49.21%, respectively. If the herd in the model was determined to be MAP negative, the model simulated the amount of milk produced, which was milk free of MAP. Therefore, we simulated the reduction of MAP contamination as a consequence of the dilution of MAP in milk silo ($MAP_{industry}$). In Figure 22, the probability density of silo milk contamination is shown for each scenario of paratuberculosis prevalence. The dilution reduced the concentration of MAP significantly. Mean concentrations of MAP were 0.08 (0.04 SD), 0.14 (0.06 SD) and 0.18 (0.07 SD) log CFU per litre of milk in silo, respectively.

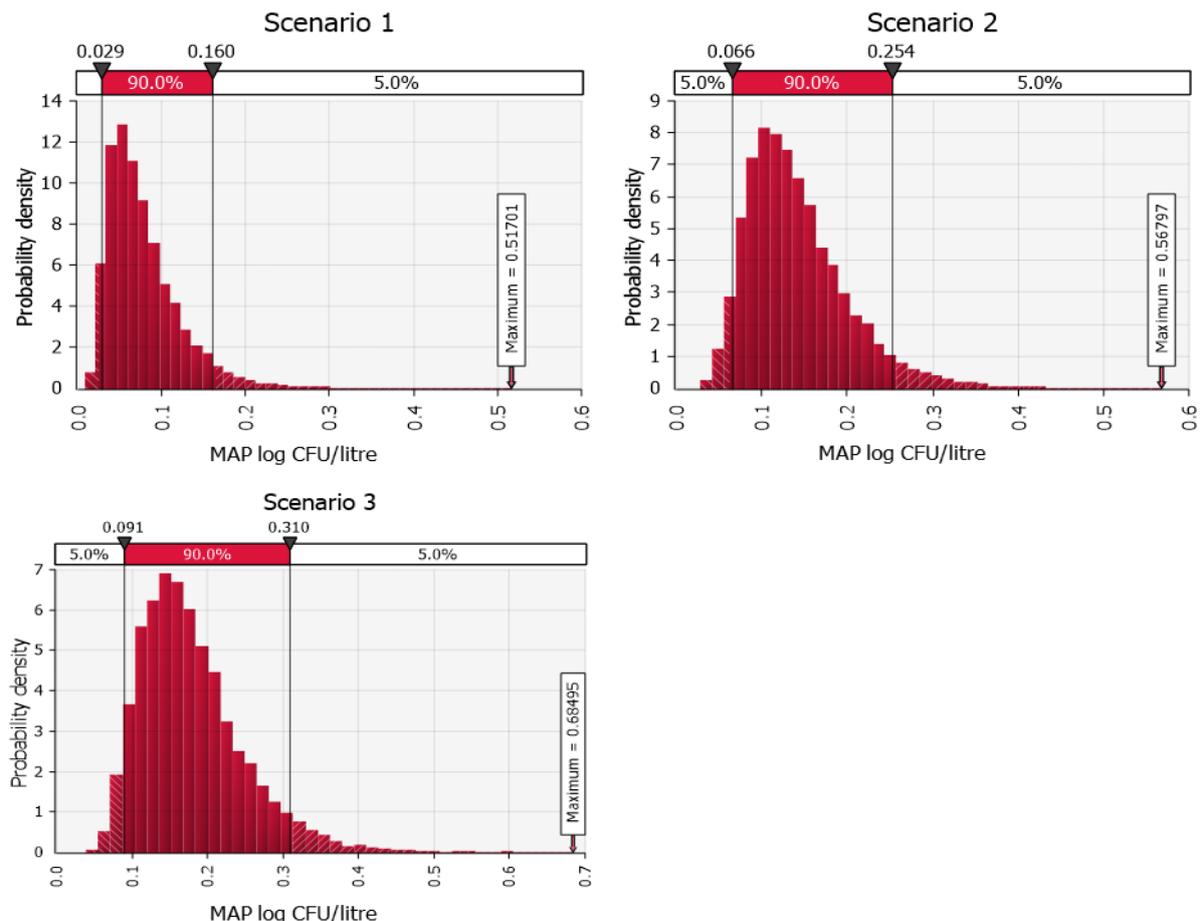


Figure 22: MAP contamination of pasteurised milk at the dairy industry level using three scenarios of mean paratuberculosis prevalence; scenario 1 with 21.3%, scenario 2 with 38.89% and scenario 3 with 49.21% prevalence.

The pasteurisation process additionally reduced MAP contamination of milk for 4 to 7 log CFU. The result of the pasteurisation and modelling MAP concentration per litre of milk was that not a single litre of pasteurised milk at the industry level ($MAP_{industry\ past}$) turned out to contain MAP cells.

It is believed that the UHT treatment of milk deactivates MAP completely (ACMSF, 2000), hence there is 0 MAP CFU per litre of UHT milk. Therefore, the contamination of UHT milk was not included into the simulation model.

4.2.3.3 Risk of exposure to MAP via milk for the average consumer

In Slovenia in the year 2018, the average annual consumption of all types of milk combined was 43 litres per household member.

The risk of exposure to MAP is highest when consuming raw milk. If for example, the consumer buys milk at a MAP positive farm or the farmer and his family are drinking that same raw milk, there is a 86.67% chance that the litre of milk they consume contains MAP cells and less than a 1.82% chance that there are more than 100 MAP CFU per litre of milk. Specifically, there is a 100% chance that they will be exposed to viable MAP in a year. But if milk at the MAP positive farm is pasteurised there is only 2.97% chance of exposure to MAP via milk in a year and none of the contaminated litres of milk would contain more than 2 MAP cells.

At the dairy industry level, in all three scenarios of herd level prevalence, our model showed that there is a zero chance to be exposed to MAP through pasteurised or UHT milk. Even though the milk in a silo was contaminated, the dilution reduced the concentration of MAP sufficiently to allow the pasteurisation process to mitigate the contamination.

Based on the data on the consumption of milk on farms and direct sale of raw milk as well as market shares of retail pasteurised and UHT milk, assuming that the average consumer consumes all these types of milk, we calculated that the average Slovenian consumer consumes 3.24 litres of raw milk, 3.94 litres of pasteurised retail milk and 35.82 litres of UHT milk. Since the chance of exposure to MAP via raw milk from positive farms was high, when accounting for shares of different types of milk on the market, and random distribution of positive herds, there was a 21–49% chance of being exposed to a small amount of viable MAP through milk in Slovenia.

4.2.3.4 Sensitivity analysis

The results of the sensitivity analysis at the farm level are shown with a tornado plot (Figure 23). No matter the method of ranking the input variables, whether it was based on the change of mean of the raw bulk tank milk contamination, regression coefficients, correlation coefficients or on contribution to the variance, the five most important input variables at the farm level were always the same. Inputs with the greatest impact on raw milk contamination were the number of dairy cows per herd (Spearman's $\rho = -0.55$), filter efficiency (Spearman's $\rho = -0.35$), MAP shed in milk by subclinical cows (Spearman's $\rho = 0.30$), the number of subclinical cows in a small herd (Spearman's $\rho = 0.26$) and MAP from faeces per subclinical cow (Spearman's $\rho = 0.14$). All other input variables contributed 0.1% or less to the variance in raw bulk tank milk contamination with MAP. The detailed impact of the number

of dairy cows per herd on the raw milk contamination with MAP is shown in Figure 24, where the cut-off value for the small herd (50 cows) can be observed.

In all three scenarios at the industry level, the amount of milk produced by subclinical cows at MAP positive farms contributed the most to the contamination of milk. The example of a tornado plot is shown for scenario 1 in Figure 25.

Additionally, we tested the influence of cut-off values for the small herd size on the outputs of the model (see chapter 3.2.3.1, paragraph four for detailed explanation). We tested four different cut-off values based on the proportion of herds presented by small herds. Specifically, 25 cows presented 79.8% of all herds, 36 cows 90%, 75 cows 98.4% and 100 cows presented 99.3% of all herds. The results are reported in Table 12 with the mean, 5th and 95th PC for milk contamination and for human exposure with the proportion of MAP positive litres of milk. In general, lower cut-off values resulted in higher milk contamination with MAP and slightly higher human exposure at the farm level but did not affect the risk of human exposure to MAP via retail milk.

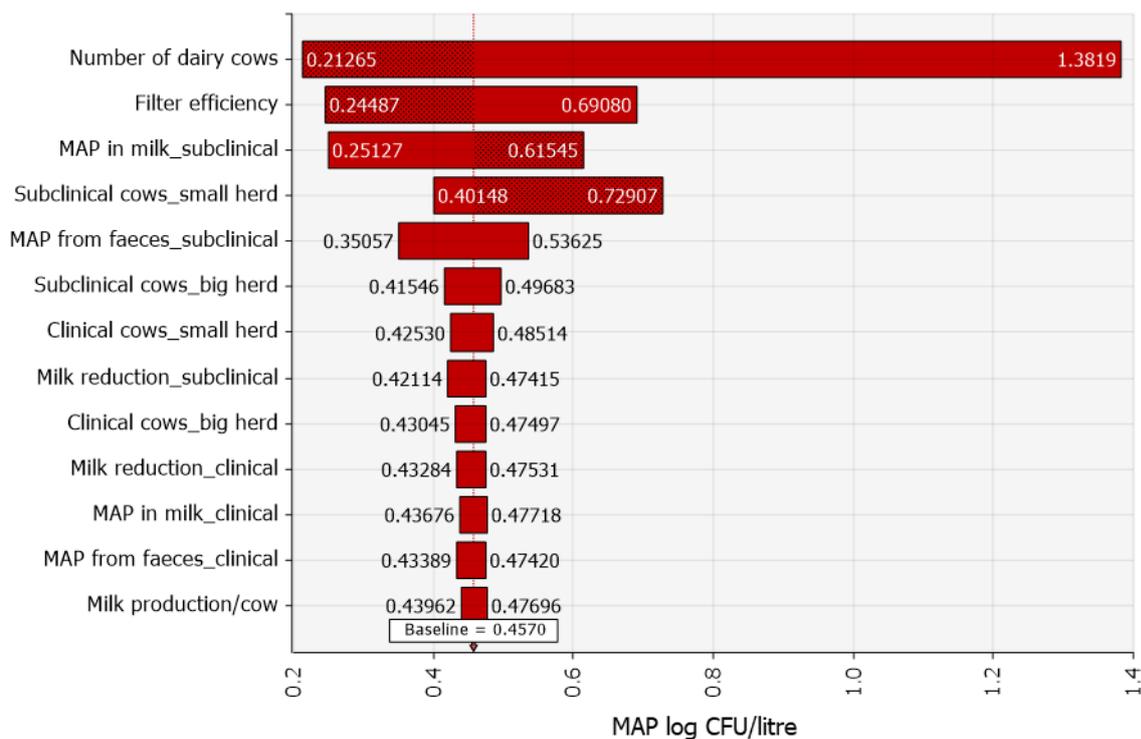


Figure 23: Input variables ranked by the effect on the mean raw bulk tank milk contamination with MAP.

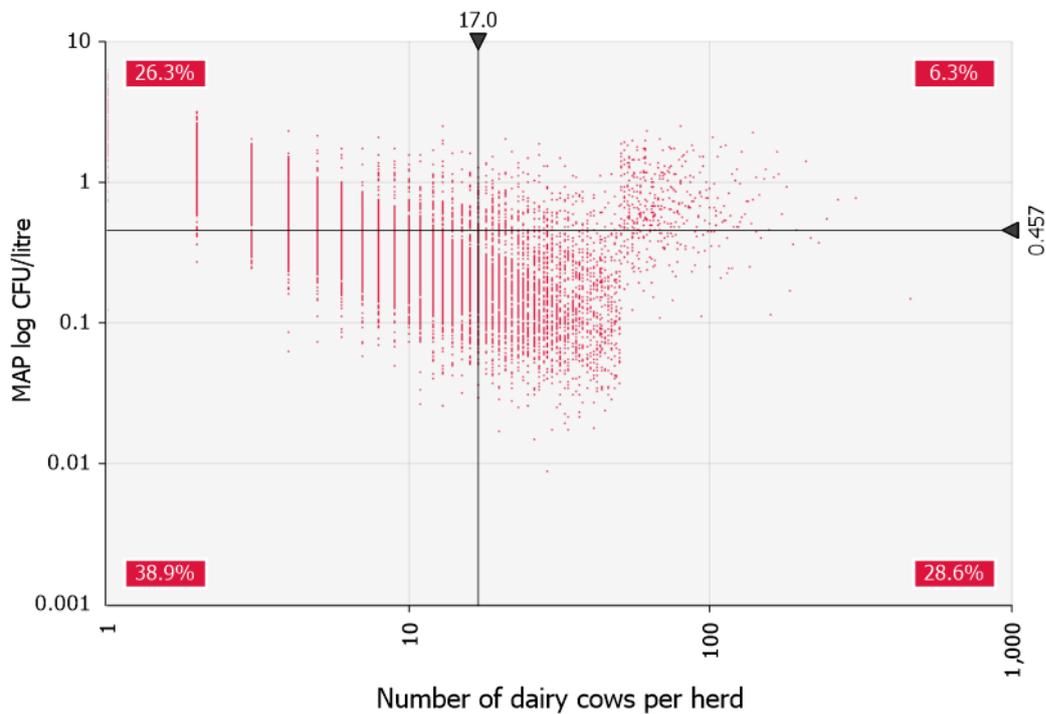


Figure 24: Correlation between MAP contamination of raw bulk tank milk and the number of dairy cows per MAP positive herd.

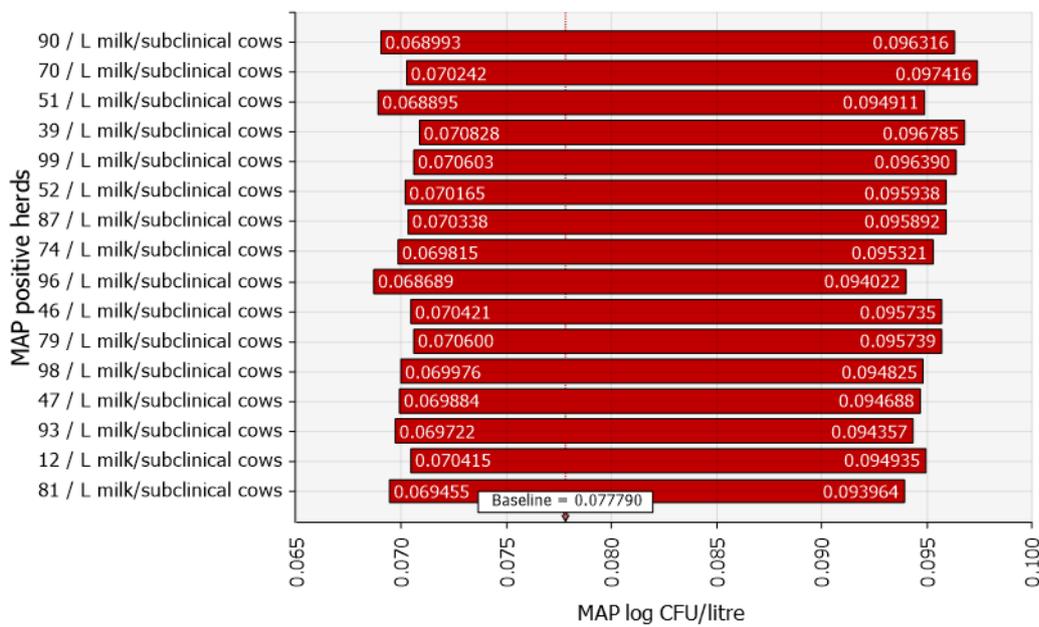


Figure 25: Input variables ranked by the effect on the mean MAP contamination of silo milk in scenario 1.

Table 12: Outputs of the human exposure assessment from the sensitivity analysis of cut-off values for the small herd size.

	Cut-off value for the number of cows in a small herd				
	25 cows	36 cows	Basic model 50 cows	75 cows	100 cows
Milk contamination	Mean (5 th , 95 th PC)	Mean (5 th , 95 th PC)	Mean (5 th , 95 th PC)	Mean (5 th , 95 th PC)	Mean (5 th , 95 th PC)
<i>MAP_{herd}</i> (log CFU/litre)	0.53 (0.1, 1.45)	0.48 (0.08, 1.37)	0.46 (0.07, 1.31)	0.43 (0.07, 1.28)	0.43 (0.07, 1.28)
<i>MAP_{herd raw}</i> (CFU/litre)	175.28 (0, 28)	166.55 (0, 24)	164.75 (0, 22)	132.12 (0, 20)	136.53 (0, 20)
<i>MAP_{herd past}</i> (CFU/litre)	0.001 (0, 0)	0.001 (0, 0)	0.001 (0, 0)	0.0008 (0, 0)	0.001 (0, 0)
<i>MAP_{industry scenario 1}</i> (log CFU/litre)	0.11 (0.04, 0.21)	0.09 (0.03, 0.18)	0.08 (0.03, 0.16)	0.07 (0.03, 0.14)	0.06 (0.03, 0.12)
<i>MAP_{industry scenario 2}</i> (log CFU/litre)	0.2 (0.1, 0.34)	0.17 (0.08, 0.29)	0.14 (0.07, 0.25)	0.12 (0.06, 0.22)	0.11 (0.06, 0.2)
<i>MAP_{industry scenario 3}</i> (log CFU/litre)	0.26 (0.14, 0.42)	0.21 (0.11, 0.36)	0.18 (0.09, 0.31)	0.15 (0.08, 0.27)	0.14 (0.08, 0.24)
<i>MAP_{industry past}</i> (CFU/litre)	0 (0, 0)	0 (0, 0)	0 (0, 0)	0 (0, 0)	0 (0, 0)
Human exposure	%	%	%	%	%
<i>Raw milk at positive farm with > 0 MAP CFU/litre</i>	89.13	87.58	86.67	85.98	85.82
<i>Raw milk at positive farm with > 100 MAP CFU/litre</i>	2.05	1.90	1.83	1.82	1.84
<i>Pasteurised milk at positive farm with > 0 MAP CFU/litre</i>	0.09	0.09	0.08	0.06	0.06
<i>Pasteurised milk at industry level with > 0 MAP CFU/litre</i>	0	0	0	0	0

4.3 RISK CHARACTERIZATION

4.3.1 Level of risk

The objective of this thesis was the risk assessment of exposure to MAP via milk and milk products for the Slovenian consumer. The results of exposure assessment, with respect to the underlying assumptions and uncertainties, show that the risk is not negligible.

Even though there are many studies about MAP presence in different types of milk and milk products, we were able to quantitatively estimate the risk only for raw milk, whole pasteurised milk and UHT milk. There were not enough data available to quantitatively estimate the probability and quantity of MAP in other types of milk and milk products. Nevertheless, we believe that the results can be extended, at least in part, to other milk products at the respective level of production. The summary of our results is shown in Table 13.

At a MAP positive dairy herd, the concentration of MAP per litre of raw bulk tank milk was relatively low, but MAP was present in a large proportion of produced milk, making the likelihood of human

exposure almost certain. The amount of MAP in milk was significantly lowered with the pasteurisation process which made the exposure unlikely, meaning that it could still occur at some point. We estimate that for those who consume raw milk and milk products from farms with paratuberculosis the risk of exposure to MAP is high. Given the popularity of milk vending machines with raw milk and home-made dairy products in Slovenia, we believe that this risk should not be ignored, especially for people with pre-existing conditions and infants. To reduce the risk, consumers should heat raw milk before consumption.

Table 13: Summary of the risk characterization by type of milk and overall exposure of the average Slovenian consumer

	Likelihood of exposure	Level of MAP CFU/litre of milk	Level of risk	Level of uncertainty
Raw bulk tank milk at a MAP positive farm	Almost certain	High	High	Low to moderate
Pasteurised milk at a MAP positive farm	Unlikely	Low	Low	Low to moderate
Pasteurised milk at the dairy industry level (all three scenarios)	Rare	Insignificant	Negligible	Low to moderate
UHT milk at the dairy industry level	Insignificant	Insignificant	Negligible	Low
Exposure of the average Slovenian consumer	Possible	Low	Low	Moderate

On the other hand, the exposure to MAP via milk collected and processed by the dairy industry is most likely insignificant. The dilution of MAP concentration in milk silo, where milk from different dairy farms is mixed together, was sufficient for MAP cells to be inactivated by pasteurisation. Since other milk products undergo further processing of milk and since MAP does not multiply outside the host, we can assume that the exposure to MAP via milk and milk products at the dairy industry level is rare. This means that it may occur in exceptional circumstances.

Based on the amount of consumption and the market share of different types of milk, we determined that the likelihood of exposure to MAP for the average Slovenian consumer in one year is possible, meaning that it might occur or should occur at some point. The level of MAP to which the average consumer might be exposed to is low; therefore, we estimated that the overall risk of exposure is low.

4.3.2 Assumptions and uncertainties

The main pitfalls of the conducted risk assessment are the assumptions of a closed market since we excluded the import and export of milk and milk products, random selection of farms that contribute milk to a dairy plant and the existence of an average consumer.

In the year 2018, Slovenia was a net exporter of milk and acidified milk, while it was a net importer of other dairy products. The overall production of drinking milk was 168,000 tons. In the same year, almost 307,000 tons of milk were exported and only a little over 44,000 tons were imported. In 2018, almost 44,000 tons of acidified milk were produced, of which 16,000 tons were exported, and 13,000

tons were imported. There were only around 16,500 tons of cheese produced. More than 10,000 tons of cheese were exported, while almost 27,000 tons were imported. The production of other dairy products was much lower and the import mostly exceeded the export. These numbers suggest that for a more accurate estimation of risk of exposure to MAP via milk and milk products for the Slovenian consumer, the posed risk should also be studied for each importing country. But in doing so, one would face similar uncertainties and lack of data and would need to establish certain assumptions as we did in our study.

The assumption of a random selection of farms that contribute milk to a dairy plant was set because we did not have data on which farms deliver milk to which dairy plant. In the real world, the delivery of milk is obviously not random. Thus, some clusters of MAP positive farms, which deliver milk to the same dairy plant, might form, either because of trade or common pastures. Depending on the herd level prevalence and the size of the milk silo at the dairy plant, it could happen that the contamination of milk in the silo would be higher than we estimated. Naturally, the opposite could also be the case.

We assumed that the average consumer exists since more detailed data were not available. The benefit of this assumption is the possibility of the estimation of the overall exposure of the Slovenian consumer but gives the results with limited practical value. That is our rationality for also showing the results and expressing the level of risk separately for each type of milk we could assess. Nevertheless, MAP is believed to pose a higher health risk for certain subgroups of the population, subsequently assessing the risk of exposure for those subgroups would be of interest.

The most important uncertainties associated with inputs of the risk assessment are the epidemiological features of MAP. Specifically, these uncertainties are: within-herd prevalence and herd level prevalence of paratuberculosis in cattle, transmission pathways in animal and human infections, infectious dose for humans or different doses for different subgroups when assuming that MAP is a zoonosis, diagnostic methods for detecting MAP and importance of exposure through milk and milk products in comparison with other possible routes of infection. To overcome these uncertainties to some extent, we used data from published literature some of which were based on empirical studies but others relayed on different models and their calibration. According to the uncertainties, we narrowed the scope of our study to assess the risk of exposure and not the risk to human health.

The most important uncertainty associated with the assessment methodology is the calibration and validation of the models with independent data since the data is not yet available. Nevertheless, this was the main reason we chose the presented approach. The models were scrutinised with thorough internal validation and sensitivity analysis as well as with consultation with the expert on MAP about used assumptions and parameters and the feasibility of the obtained results.

Considering all the information presented in this thesis it is not possible to derive the exact risk of exposure to MAP via milk and milk products for the Slovenian consumer. However, our results indicate which types of milk should cause concern if the link between MAP and human diseases is established in the future. Moreover, we identified gaps where future studies should focus their efforts in order to reduce the mentioned uncertainties.

5 DISCUSSION

5.1 WITHIN-HERD SPREAD OF MAP IN A TYPICAL SLOVENIAN DAIRY HERD

To estimate the proportion of subclinically and clinically infected cows in a typical Slovenian dairy herd infected with MAP we developed a stochastic compartmental model. The main reason for the development of this model was the lack of data on within-herd MAP prevalence in Slovenia since the last study was conducted in the year 2008 in three dairy herds. Another issue besides the small sample size and time of the study is that the prevalence was studied in big herds, which is not a good representation of the dairy herd structure in Slovenia. In the study on herd and animal level prevalence, Ocepek et al. (2002) argued that the low animal level prevalence in Slovenia could be partially explained with the existence of numerous small herds since the spread is more probable within than between herds. We hypothesized that since the average size of farm holdings in Slovenia has increased in the last few years, mainly due to merging of farm holdings, this has provided better conditions for the spread of MAP.

We defined a typical Slovenian dairy herd as a herd with the average number of dairy cows per dairy herd in Slovenia. All possible herd demographic parameters were estimated from Slovenian data. Some of the values were supported with the expert opinion because the average value did not reflect the true conditions in a small herd. The reason for this is that average values are calculated on the animal level which makes them biased towards bigger herds. Namely, the data on farm holdings by size classes of the number of dairy cows for the last available year, that is 2016, showed that dairy herds with 20 or more cows per herd represented less than 17% of all dairy herds but were rearing almost 54% of all dairy cows in Slovenia. Thus, the average values on the animal level better reflect the situation in bigger herds than in smaller ones. The average number of dairy cows per farm was 17.1, therefore, we used 17 cows as the initial number of dairy cows in the compartmental model. However, we did not use the average number of young animals per herd in Slovenia, but the average number of young animals in herds with 17 cows. According to the Results of dairy and beef recording (Sadar et al., 2019), the average lifetime performance of dairy cows culled in the year 2018 was approximately 1318 days. In the expert's opinion, this is more a reflection of high productive herds, whereas smaller herds are less intensive and farmers tend to retain their cows longer. The estimated lifetime of a cow in a small herd was eight years, meaning six productive years, which was the value we based our culling rate on for all the cows, except the high shedding ones. The culling rate of high shedding cows was calculated based on the expert's opinion that farmers with smaller herds tend to treat their cows and hope for improvement of their health longer than those who manage more intensive herds. The number of cows introduced into the herd and the birth rate of all cows, except high shedding cows, was calculated directly from the available data. We did try to make some adjustments of these figures to better reflect the situation in a small herd, but in any of those cases, the herd size did not stay stable for the whole studied period when introducing MAP infection, since the introduction of MAP infection increased the culling rate and decreased the birth rate. Therefore, we decided to calibrate the model to assure that the herd size of a naive herd was stable for at least 20 years. After calibration of the model with MAP infection, we ended up using the values calculated directly from the available data. Additionally, the culling rate of young animals of approximately 12% of animals per year was used only if there were redundant young animals in the susceptible young and latent young compartments in order to assure enough replacement cows for holding the number of adult cows stable.

Other parameters used related to MAP transmission, disease progression and disease effect on reproduction and culling were based on data from the literature. We chose the compartmental model because on one hand there were quite a few studies published on the within-herd dynamic of MAP infections, but on the other hand, a lot of parameters were obtained only through model calibrations and expert opinions. We assumed that modelling on a compartmental level with introduced stochasticity would be sufficient enough to get an insight into the MAP within-herd dynamic and would enable the use of published parameters from different types of models. Indeed, we collected parameters from compartmental models and individual or agent-based models with different combinations of health states and transitions, paths of infections, considered herd dynamics and time steps. Daily, weekly or monthly time steps were applied in these studies. We utilized the parameters from the following studies, although we had to recalculate some values that they corresponded to the structure of our model and our time step.

Marcé et al. (2011a) developed a stochastic compartmental model of MAP within-herd spread in a dairy cattle herd with six health states, namely susceptible, transiently infectious, latently infected, subclinically infected, clinically affected and resistant. They modelled herd dynamics with represented housing facilities. Resistant animals were all uninfected animals older than one year and they did not consider infections of adult cows possible. They used the French agricultural statistics data to construct a herd, their initial herd included 114 dairy cows. However, in another study on the same model, they simulated MAP spread also in a herd with 35 adult animals, but they did not find a correlation between the herd size and the results (Marcé et al., 2011b). They accounted only for vertical and indirect transmission from calf to calf and cow to calf. All other studies we used were developed for the MAP spread within dairy herd in the United States (Martcheva et al., 2015; Mitchell et al., 2015b; Robins et al., 2015; Al-Mamun et al., 2017; Smith et al., 2017). Their herd size varied from 100 to 1000 dairy cattle. They all accounted for direct, indirect and vertical transmission as well as adult infections, however, the implementation varied. With the exception of Martcheva et al. (2015) who only modelled the calves and cows compartment, all the other researchers modelled heifers as a separate group or individuals. Robins et al. (2015) and Al-Mamun et al. (2017) developed individual or agent-based models, with three infection states: latent or exposed, low shedder and high shedding cow. Both used some parameters from the literature but used a different approach for obtaining the missing parameter values for MAP transmission. Robins et al. (2015) used expert opinions and assumptions, whereas Al-Mamun et al. (2017) assumed prevalence and obtained transmission coefficients and rates through calibration of the model. Mitchell et al. (2015b) developed a state transition model with transient, latent, low shedder and high shedder state for infected states. For estimating parameter values, they used longitudinal field data with considered MAP strains. They cautioned that even when using a large field data set, due to imprecise tests and slow disease progression, the true prevalence and incidence are likely to be underestimated. Smith et al. (2017) modelled two paths of progression of the disease, which was the basis for the development of our model. Since our main outputs of interest were the number of subclinically and clinically infected cows, we modelled calves and heifers together as young animals and calculated their infections and transmission rates accordingly.

In a study reviewing the control of paratuberculosis in 48 different countries the most often reported within-herd prevalence in dairy herds was 5–15% (Whittington et al., 2019). However, as already stated above, many studies draw attention to problem of inadequate diagnostic tests and the iceberg

phenomenon of paratuberculosis infections, some estimating that up to 70% of infected animals are undetectable (Whitlock and Buergelt, 1996). Contrary to our expectations, our model did not show a higher within-herd prevalence of MAP in Slovenia than was previously estimated (Kušar et al., 2011). Even though the average herd size increased from 12.5 dairy cows in 2008 to 17.1 dairy cows per farm in 2018⁶, this did not provide better conditions for the spread of MAP.

The logical results of our sensitivity analysis ensured that the input parameters were specified appropriately and that the model was mathematically reasonable. Additionally, it enabled the exploration of the effect of the minimum and maximum plausible values of eight epidemiologically most important parameters. It showed that the transmission rate from infected cows to young animals and from infected cows to susceptible cows were the most important parameters in determining the prevalence, as well as the proportion of subclinically and clinically infected cows per herd. This implies that infected cows on either the low or the high shedding path were the most influential compartment in the model. However, the transmission rate from high shedding cows followed closely, but the overall effect was lower because there were less clinically infected than infected cows. The proportion of latently infected young animals entering the high shedding path had a greater impact on the proportion of high shedding cows than on the other two outputs of the model. These results suggest that in the applied settings culling of only high shedding animals would have a limited effect on lowering the MAP within-herd prevalence.

To the best of our knowledge, this stochastic compartmental model is the first one specifically designed to represent a small dairy herd. Although there were some attempts to model small herds, they were not small in the context of the Slovenian herd structure but rather of medium size. Furthermore, the model was not stable when we set the initial herd size to 100 dairy cows. Our results suggest that different models should be used for studying the spread of MAP in herds of different sizes. Nevertheless, finding the threshold for the herd size was not our aim in this model. Our aim was to get the possible proportions of subclinically and clinically infected cows; however, the model could have offered more insight if it were explored in further detail. For example, we could calculate the incidence of fadeouts and reinfections and assess the endemic steady state of paratuberculosis within typical Slovenian dairy herds. The model could be further developed and even more precise data on herd structure and dynamics could be implemented; nonetheless, we believe this model showed sufficient representation of a typical Slovenian herd and enabled us to obtain the data we needed for the human exposure model. The development of this model is relevant also for other countries with a similar system since it can also serve for testing the efficiency of different control measures. This could be of great importance if a link between MAP and human diseases is established in the future.

5.2 NETWORK ANALYSIS OF CATTLE TRADE AS AN AID TO MAP CONTROL MEASURES

A static representation of the network was used for the characterization of a global and local network structure with special attention given to the epidemiological implications. By definition, the static network is an approximation of the temporal network, since it does not include the information about the time of movements and hence does not take into account the succession of movements, which is

⁶ https://pxweb.stat.si/SiStatDb/pxweb/en/30_Okolje/30_Okolje__15_kmetijstvo_ribistvo__03_kmetijska_gospod__02_15166_zivinoreja/?tablelist=true

an integral part for understanding a disease dynamic. Therefore, it overestimates the network connectedness and the outbreak size and does not provide any information about the outbreak duration (Lentz et al., 2013; VanderWaal et al., 2016). Even though there are considerable shortcomings of using static network analysis in veterinary epidemiology, it is deemed sufficient when trying to provide an overview of the network topology. Based on the current knowledge in comparison to temporal network analysis, the static analysis offers clear definitions of global and local network measures that can be fairly easily computed without the use of programming languages. For example, large animal trade networks can be analysed with the Pajek software, which can be used by non-computer experts. In fact, we used Pajek for the whole static network analysis, except for the generation of random networks and the targeted node removal. Another important benefit of using static network analysis is that the results are easily interpretable and tangible, which render them useful for planning targeted intervention measures.

To determine whether the network analysis could offer support in increasing the efficiency of paratuberculosis intervention measures in Slovenia, we used yearly snapshots of our cattle trade network to quantify year to year consistency of cattle movements and heterogeneity in the importance of premises. Monthly networks were used to assess seasonality. We chose these aggregation windows because it was previously argued that networks aggregated over longer time periods may adequately address chronic or slowly spreading diseases (Kao et al., 2007). Even though the Slovenian cattle movement network is in general smaller and less active compared to other European cattle trade networks, it shares a number of properties with them, such as similar degree distributions, average shortest path length and diameter (Natale et al., 2009; Nöremark et al., 2011; Mweu et al., 2013; Dutta et al., 2014; Vidondo and Voelkl, 2018).

In the monthly networks, two peaks in the frequency of animal movements were apparent during late spring to early summer and in autumn, which is due to the grazing period. Similar seasonal patterns were described also in other countries with grazing practices (Natale et al., 2009; Nöremark et al., 2011; Vidondo and Voelkl, 2018). In our cattle movement networks, this seasonal pattern was reflected in all monthly global network measures except diameter and was consistent through time. In addition, global and local network measures in the yearly networks showed consistency throughout the studied period. As in studies of cattle movements in other countries (Natale et al., 2009; Frössling et al., 2013; Marquetoux et al., 2016b), node level measures have also proven to be of great assistance in identifying premises that are disproportionately important from the epidemiological perspective compared to other premises in the Slovenian cattle trade network. Collection centres without exception were among these premises. The finding that this type of premises has central positions within the network and consequently poses a higher risk for disease spread is consistent with results from other studies (Mweu et al., 2013; Vidondo and Voelkl, 2018). As expected, pastures and mountain pastures were also shown to be important for disease spread. This is substantiated with the circumstances of the grazing system since animals stay at the designated location for a longer period and can homogeneously mix with all other animals at the same location, both of which do not hold for collection centres, exhibitions or fairs. Since MAP can be shed in faeces in high quantity and the main route of infection is a faecal-oral route, pastures provide ideal conditions for disease spread.

Even though the network measures were consistent over time, year to year contacts between premises turned out to be preserved only to some extent. The same conclusion was reached by

Marquetoux et al. (2016b) with the evaluation of the consistency of contacts between pairs of farms. We have observed this in three instances: First, with the calculation of yearly changes in premises affiliation to components of the bow-tie partition (Figure 13). Second, with correlations in network measures between different years. And third, by targeted node removal (Figure 17). The latter was used to test whether the local network measures could increase the efficiency of intervention measures. We simulated a risk-based selection of premises in the last studied year based on premises ranking in the previous years and tracked changes in network connectedness when individual premises were removed. The results showed that disassociation of the network was substantially improved when the node removal was informed and the results were increasingly better when using more recent information. In our case, the degree centrality was the most effective measure in reducing the size of GSCC; however, the simulation of the targeted node removal in other years would be needed to determine if this was changing through time. Korschake et al. (2013) showed that nodes identified as the most important in a static network also remained relevant in the temporal network approach.

In this thesis, we presented the first in-depth analysis of the Slovenian cattle trade characterized as a complex network. We argue that network analysis may aid in the optimization of paratuberculosis surveillance and control in Slovenia. When providing advice, the latest available data should be used. The results of this study are applicable for risk-based interventions for other cattle diseases that spread mainly through animal movements.

5.3 POSSIBLE MAP SPREAD BETWEEN HERDS

For the evaluation of a possible disease spread through cattle movements, we used the full temporal resolution of the network. This was modelled in terms of a temporal network, where the causal accessibility is captured and consequently, we obtained a more realistic representation of possible disease dynamics. The SI model presented herein shows possible MAP spread based on the empirically estimated true prevalence of paratuberculosis in Slovenia and four scenarios for different transmission probabilities. These simulations allowed for an estimation of the probability distributions of paratuberculosis prevalence at any time during the studied period. Nevertheless, the objective was not to get the exact prediction of paratuberculosis prevalence, but rather to study the implications of network structure for MAP spread. Given the low infection rate and the slow course of the disease, within-herd dynamics should be included. At least herd demographic data and the probability of infection on the animal level should be implemented when using the temporal network model for a reliable prediction of disease prevalence. Therefore, our model overestimates the disease spread and represents a worst-case outcome.

Based on the results from the static network analysis and the relatively high proportion of initially infected herds, the resulting high prevalences at the end of the study period were expected. The within-herd prevalence and the probability of moved animals being infected had a significant influence on the velocity of disease spread through the network. The peaks in daily incidence rates of newly infected herds were observed at the end of the grazing period (Figure 19). Since the peaks were not present in the first autumn of any scenario, in the best-case scenario and at the end of the worst-case scenario, the threshold in the number of infected premises of a certain type appeared to exist. These crucial premises were farm holdings with common pastures. At the beginning of the simulation, the

threshold was not reached because the initially infected premises were randomly selected. In the worst-case scenario, the disease reached the majority of these premises within the first three years.

Our model did not include the possibility of the infection fading out, but this was regarded as a reasonable assumption since the extinction of paratuberculosis at the population level without the implementation of effective control measures is unlikely to occur (Beaunée et al., 2015). We are aware that this is an oversimplified model, but we are focusing on the phase of spreading where more complex mechanisms, such as countermeasures or recovery, are not yet established since the disease has a slow onset and there are no control measures in place in Slovenia. In addition, studies suggest an increase in paratuberculosis prevalence in most countries with a significant dairy industry (Barkema et al., 2010). The high increase in paratuberculosis prevalence is supported by a number of recent studies. Whittington et al. (2019) reported that from 48 countries included in the survey approximately half had more than 20% of herds infected. In some developed countries (e.g. Italy, France, Germany, the United Kingdom) prevalence exceeded 40%. Even though the reported prevalences were high, the authors warned that underreporting and underestimating paratuberculosis prevalence are both common.

The model included a couple of underlying assumptions that have probably contributed to an overestimation of the transmission potential. We did not distinguish between animals based on sex, age or breed and thus, the risk each animal posed for disease transmission was assumed the same. We did not consider the time an animal spent at individual premises. For example, if an infected animal was introduced to a farm holding and the next day the same holding sold an animal, the probability that it was infected was the same as for the animals sold months after the introduction of the infected animal in the herd. It was assumed that all the animals at the same premises have equal chance to get infected, which for example excludes the possibility that animals at the same farm holding of different categories were housed in different buildings or that animals at an exhibition were effectively separated. Another limitation is that we did not include import or export movements and regarded our network as a closed system. Imports were not considered since we only had the data on the country from which the animal was imported and thus the model would assign the same health state to the whole country.

On the other hand, we adopted some assumptions that probably led to an underestimation of the potential disease transmission. The period in which the study of paratuberculosis prevalence was conducted and the starting date of our network are 2.5 years apart. Therefore, the proportion of initially infected herds was probably higher than the one we used in the model. As the prevalence in this study was calculated, we did not have data on which herds were infected and therefore used random sampling, which may affect the result. With the exclusion of end nodes from the network, we adopted the assumption that animals moved to slaughterhouses, dead animals or their parts do not pose any risk for the transmission of disease. This may not be the case for MAP, but the spread through these routes is perceived to be unlikely. Furthermore, we inherently neglected other potential transmission pathways; for example, movement of other susceptible or non-susceptible animal species, movement of owners, workers or veterinarians, transmission via vehicles, shared equipment or bioaerosol (Eisenberg et al., 2012; Rossi et al., 2017b). The main reason was the lack of empirical data and the fact that tackling direct transmission of MAP through cattle movements should be the first step in the efforts to control the spread between herds.

Even though the SI model was based on several simplifying assumptions, it offered an insight into the importance of between-farm movements for disease propagation in the cattle population. Moreover, the employed approach with diluted networks may also be used to model other between-herd transmission pathways.

5.4 RISK ASSESSMENT OF HUMAN EXPOSURE TO MAP IN MILK AND MILK PRODUCTS

The ultimate aim of this thesis was to assess the risk of exposure to MAP via milk and milk products for the Slovenian consumer. The main reason for this is the increasing concern for public health and the ongoing efforts to assess and subsequently lower human exposure to pathogens through the food of animal origin. The second reason is the importance of the dairy sector's contribution to the Slovenian agricultural industry since the milk output accounts for almost a third of the whole livestock output (Bedrač et al., 2018). Paratuberculosis is known to cause significant direct disease losses in the cattle industry (Barkema et al., 2010; Cho et al., 2013) and consequently puts pressure on one of the most important parts of the Slovenian agriculture. In addition, the cattle industry may be subject to indirect disease losses due to possible MAP related trade bans. In 2018, Slovenia exported for more than EUR 169 million of milk and milk products and imported for more than EUR 173 million⁷. We presumed that the prevalence of paratuberculosis in Slovenia is now higher compared to the estimates from a decade ago and thus aimed at assessing the possible prevalence as well as subsequent risk for human exposure despite the lack of empirical data.

Numerous studies of empirical estimation or modelling the occurrence of MAP in milk and milk products have been published. However, no such estimates have been attempted in Slovenia. Since our compartmental model indicated that there may be an important difference between modelling small or large herds we developed a stochastic quantitative risk assessment model with Monte Carlo simulations to estimate the level of milk contamination and the amount of produced milk in the MAP infected Slovenian dairy herds. The most important assumptions and uncertainties were discussed in section 4.3.2 under Risk characterization.

For the input variables based on Slovenian data in our previous two models, we selected distributions using the AIC selection criterion. Other input variables were based on the literature and expert opinion; subsequently, simpler probability distributions were applied such as triangular and uniform. The exceptions were probability distributions for the indirect contamination of milk with MAP from faeces and MAP reduction with pasteurisation where we used unchanged distributions from previous studies. For the indirect contamination of milk with MAP from faeces, separately for subclinically and clinically infected cows, we applied distributions used by Beaunée et al. (2015). Another possible way of modelling indirect MAP contamination of milk was by modelling MAP shedding in faeces, the contamination of milk with faeces or dirt and calculating the indirect contamination of milk with MAP from faeces or dirt. In the expert's opinion, making the model simpler was a better option since there were no precise data available and we did not have any estimations for these steps from the Slovenian data. We used the same distribution as Serraino et al. (2014) for the MAP reduction with pasteurisation since we found their argument justified. They explained that they adopted a conservative approach

⁷ https://pxweb.stat.si/SiStatDb/pxweb/en/20_Ekonomsko/20_Ekonomsko__24_zunanja_trgovina__01_lzvoz_in_uvoz_bлага__02_Podatki_KN__8-mestni_KN/

because even though the reduction of more than 8 log was reported, still viable MAP was found in some experiments.

Based on the expert's opinion, we modelled the proportion of subclinically and clinically infected animals in a herd in two ways. If the model simulated the number of cows that corresponded to a small herd, the proportions of infected animals were calculated based on the results of our compartmental model; otherwise, the proportion was calculated based on data obtained from the literature. This also reflects one of the conclusions of our compartmental model, arguing that within-herd MAP spread dynamics is dependant of the herd size. Also, since the results of the compartmental model showed very weak correlation between the proportion of subclinically and clinically infected cows, we kept these two input variables in the exposure assessment model independent.

In the model, the cut-off value for a small herd was set to 50 cows. We tested the influence of the cut-off value on the results of the model and found that lower cut-off values resulted in slightly higher human exposure at the farm level but did not affect the risk of human exposure to MAP via retail milk in any of the prevalence scenarios. The higher exposure at the farm level when applying lower cut-off values is a consequence of using a probability distribution for bigger herds in a higher proportion of herds, since we assumed that the prevalence of MAP is higher in bigger herds. Similarly, the cut-off value influenced the correlation between the number of dairy cows per herd and MAP concentration in raw bulk tank milk. A cut-off value of 50 cows led to a moderate negative correlation, while 25 cows lowered the correlation to weak (Spearman's $\rho = -0.22$). The negative correlation was not surprising since we modelled MAP contamination of milk in a positive herd, which means that the minimum number of infected cows was one and in small herds, one animal represents a higher proportion of the herd. When accounting for the number of infected animals per herd, meaning the prevalence, the relationship between prevalence and MAP contamination of raw bulk tank milk was monotonous and positive for all tested cut-off values.

In the sensitivity analysis, next to the number of cows per herd, the other important input variables were milk filter efficiency and variables connected to subclinically infected cows. For milk filter efficiency we assumed uniform distribution from zero to the value used by Rani et al. (2019) based on Van Kessel et al. (2008) which studied the faecal prevalence of *Salmonella*. The rationale for modelling this range of values was that MAP is small and may pass a filter; nevertheless, it tends to form clumps which could be removed by a filter, but we had no data on the situation in Slovenia. Subclinically infected cows and their internal and external contamination of milk is more important than variables related to clinically infected cows because they are more prevalent and produce more milk. Therefore, if one wanted to reduce the amount of MAP in raw bulk tank milk, the culling of clinically infected cows would not be sufficient.

Our results are in agreement with conclusions of other studies (Cerf et al. 2007; Weber et al., 2008; Boulais et al., 2011; Okura et al., 2013) that the required pasteurisation time and temperature regimes are sufficient to inactivate possible MAP cells contaminating milk at the dairy plant. Cerf et al. (2007) stated that studies reporting the number of MAP cells in retail milk did not display probable contamination of raw milk. They also proposed explanations for the detected level of MAP, which were improper pasteurisation and cross-contamination of samples at the laboratories or contamination of pasteurised milk with raw milk during milk processing at the dairy plant. However, our result of the

proportion of contaminated litres of raw milk at the farm level showed a large proportion of milk containing viable MAP cells. After pasteurisation of milk at the dairy herd level MAP was largely inactivated, but still not entirely. It is suggested that even a small number of MAP cells could be responsible for triggering excessive immune response and thus induce Crohn's disease in some patients. Therefore, even though we concluded that the risk of exposure to MAP via milk and milk products in Slovenia is low, our results should cause some concern. This is especially true when considering people with pre-existing conditions and infants, since the consumption of raw milk and homemade milk products in Slovenia is quite common. Vulnerable groups should avoid drinking raw milk or heat the raw milk before consumption.

The results of our risk assessment of human exposure to MAP could provide support to decision-makers in Slovenia if the link between MAP and human diseases is confirmed and risk management would then be needed. Despite all the underlying assumptions and uncertainties in this quantitative risk assessment, we believe the results could serve as a good start for possible risk mitigation efforts. Lastly, it should be stressed that our results could be applicable to other countries or regions with a similar dairy herd structure.

6 CONCLUSIONS

Hypothesis 1: The risk of human exposure to MAP by consumption of milk and milk products in Slovenia is not negligible.

- The results of our exposure assessment model, with respect to the underlying assumptions and uncertainties, showed that the overall risk of exposure to MAP via milk and milk products for the Slovenian consumer is low.
- For consumers who drink raw milk and consume raw milk products from farms with paratuberculosis the risk of exposure to MAP is high. However, the simulated amount of MAP per litre of raw bulk tank milk was relatively low.
- The risk of exposure to MAP via milk collected and processed by the dairy industry is most likely insignificant.

Hypothesis 2: The developed compartmental model will show higher prevalence of MAP in Slovenia compared to previous estimates.

- The developed compartmental model did not show higher prevalence of MAP in Slovenia compared to previous estimates.
- To the best of our knowledge, our stochastic compartmental model is the first one specifically designed to represent a small dairy herd and could be applicable to other countries with a similar dairy farm structure.

Hypothesis 3: The analysis of Slovenian cattle movements will show that, in order to reduce MAP spread and consequently reduce the human exposure via milk and milk products, it would be particularly useful to implement intervention measures on premises with higher centrality measures.

- The analysis of Slovenian cattle movements showed that in order to reduce MAP spread, it would be particularly useful to implement intervention measures on premises with higher centrality measures.
- We presented the first in-depth analysis of the Slovenian cattle trade characterized as a complex network.
- The employed approach of modelling between-herd spread of MAP with diluted networks may also be used to model other between-herd transmission pathways or other diseases.

Additional scientific contribution:

- We identified gaps where future studies should focus their efforts. These are mainly connected to MAP epidemiological features.
- This thesis showed that comprehensive insights into MAP spread and possible human exposure can be reached despite the lack of empirical data.
- Our results could provide support for informed decision-making in MAP intervention measures.

7 SUMMARY

In cattle, *Mycobacterium avium* subsp. *paratuberculosis* (MAP) causes paratuberculosis or Johne's disease, a worldwide endemic disease with large economic consequences. It may as well represent a risk for human health. Potential sources of human exposure are food (e.g. milk, meat), water, environment and direct contact with infected animals. Our objective was to assess the risk of exposure to MAP via milk and milk products for the Slovenian consumer.

To meet the objective of our study, we used a modified risk analysis framework outlined by the World Health Organization and the Food and Agriculture Organization of the United Nations in the Risk Characterization of Microbiological Hazards in Food (2009) combined with the guidelines of the World Organisation for Animal Health in the Handbook on Import Risk Analysis (Volume I and II, 2004). We performed the first two components of the risk analysis, namely hazard identification and risk assessment, whilst risk management and risk communication were beyond the scope of this thesis.

In the hazard identification step, we examined the characteristics of MAP, which were important for the development of our models and the potential risk it may pose for human health. MAP is a small, very slow growing bacterium with a thick lipid-rich cell wall and tends to form clumps. The wall renders MAP acid-fast, hydrophobic and highly resistant to chemical and heat treatment. It is a multi-host pathogen, with the main reservoir and host probably being livestock ruminants. It can infect and cause clinical signs in numerous other domestic and wild animal species, including non-human primates. Besides the gastrointestinal tract, it can be detected in many other tissues during systemic infection. MAP is believed to be unable to multiply outside the host, however it can survive in the environment for more than a year. Infected cattle, already in the subclinical phase, may shed large quantities of MAP in milk and faeces. The latter is the main source of environmental contamination and the spread of the pathogen.

There are reasonable grounds to suspect that MAP may present a risk for human health. It is assumed to be an infectious agent of Crohn's disease and some other chronic diseases. To the best of our knowledge, there is still no evidence confirming the causality. Nevertheless, it is important to improve the understanding of the risk of human exposure. Viable MAP were found in raw bulk tank milk, pasteurised retail milk, retail cheese from pasteurised and raw milk and powdered infant formula.

We performed quantitative risk assessment and used modelling to overcome the lack of empirical data. We developed a compartmental model to study within-herd spread of MAP and the spread of MAP on a temporal network model to study the possible spread between herds in Slovenia. The results were used in the final model which was a stochastic quantitative exposure assessment.

The stochastic compartmental model of MAP spread in a typical Slovenian dairy herd was developed using the Epidemiological Multi-Level Simulation Framework, which utilizes finite state machines. Compartments represent different health states that capture the course of the disease. We modelled the low and high shedding path of infection, with adult cows divided into six possible health states, with one susceptible and five infection stages. We added compartments for young susceptible animals, young latent animals, culled animals and one for newly introduced animals into the herd. All parameter values were deterministic but rates were automatically converted into probabilities thus introducing

stochasticity. Parameters on herd structure and demography were estimated based on Slovenian data obtained from the Agricultural Institute of Slovenia (KIS) and the Statistical Office of the Republic of Slovenia (SURs) and referred to the year 2018. Parameters regarding MAP epidemiological features were obtained from the published literature. Some values were adjusted based on expert opinion and we calibrated the model to make the herd size stable. The infection started with the introduction of one infected animal into the naive herd. Then, the within-herd spread was simulated for 10 years with a daily timestep and 1,000 iterations. We performed internal validation of the model and sensitivity analysis on the epidemiologically most important parameters.

Contrary to our expectations, the model did not show a higher within-herd prevalence of MAP in Slovenia than was previously estimated. Even though the average size of farm holdings in Slovenia increased in the last few years (from 12.5 dairy cows in 2008 to 17.1 dairy cows per farm in 2018), this did not provide better conditions for the spread of MAP. Since we developed this model to provide data for the exposure assessment model, we calculated the prevalence, proportion of subclinically and clinically infected cows, only concerning the adult animals. The results showed the mean within-herd prevalence to be 7.13% with 8.43% standard deviation (SD), 5.56% median and 70.59% maximum prevalence. There were on average 0.47% (1.63% SD) clinical and 4% (5.42% SD) subclinical animals with a median for both being 0%.

The results of our compartmental model suggest that different models should be used for studying the spread of MAP in herds of different sizes. To the best of our knowledge, this stochastic compartmental model is the first one specifically designed to represent a small dairy herd. Our model could be useful also to other countries with a similar dairy farm structure since it can also serve for testing the efficiency of different control measures.

We explored the structure of the cattle trade network in Slovenia and evaluated the potential for MAP spread through movements utilizing network analysis. The data on cattle movements were obtained from the Administration of the RS for Food Safety, Veterinary Sector and Plant Protection. We used the data from August 2011 to July 2016. As we were interested in movements that are epidemiologically important for the spread of disease, we omitted all movements to premises that corresponded to the end nodes. We performed a static network analysis on monthly and yearly snapshots of the network using Pajek software and targeted node removal using R package igraph. Additionally, we simulated MAP spread with a susceptible-infectious (SI) model on the temporal network using Python programming language.

The results showed consistency in the network measures over time, nevertheless, year to year contacts between premises tended to change. The importance of individual premises for the network connectedness was highly heterogeneous. The most influential premises in the network were collection centres, mountain pastures and pastures. Targeted node removal based on the ranking of local network measures from previous years was substantially more effective in network disassociation than random node removal. The SI model was based on the empirically estimated true prevalence of paratuberculosis in Slovenia and four scenarios with transmission probabilities of 0.02, 0.15, 0.25 and 0.40. At the end of the study period, the predicted herd prevalence obtained with 1,000 realizations for our chosen transmission probabilities were 21.30%, 38.89%, 49.21% and 59.85%, respectively.

In this thesis, we presented the first in-depth analysis of the Slovenian cattle trade characterized as a complex network. The employed approach of modelling between-herd spread of MAP with diluted networks may also be used to model other between-herd transmission pathways or other diseases. Our results show that network analysis may aid in the optimization of paratuberculosis surveillance and control in Slovenia.

To estimate the potential human exposure to MAP via milk and milk products in Slovenia we performed a stochastic quantitative risk assessment with Monte Carlo simulations using @RISK programme. We simulated MAP contamination of raw milk and pasteurised whole milk at the farm and dairy industry level and possible human exposure. We used the data obtained from SURS, KIS, the Agency of the RS for Agricultural Markets and Rural Development, the Chamber of Commerce and Industry of Slovenia and literature as well as an expert opinion. All used data referred to the year 2018. We fitted distributions to the data on demography and production at the farm level as well as to the results of our previous two models and selected distributions with the minimum Akaike Information Criterion value. The estimation of probability distributions for the reduction in milk production, MAP contamination and efficiency of milk filter were informed by literature and expert opinion. If a small herd was simulated, the proportion of clinical and subclinical cows per farm was calculated based on the results of our compartmental model, otherwise, the distribution was based on published data. A small herd was defined with the cut-off value of 50 dairy cows. The collection of milk by the dairy industry was modelled using 100 farm level models and three scenarios for the probability of the herd infection status, which were based on the temporal network model while excluding the worst-case scenario. We simulated 10,000 iterations of Monte Carlo simulations using a Latin hypercube sampling method. Additionally, we performed a sensitivity analysis and tested different cut-off values for a small herd.

Our results show that in case the farm is infected there is a high probability that raw bulk tank milk - and thus raw consumption milk at the farm level - is contaminated with MAP. Specifically, there is more than 85% chance that the litre of milk contains MAP cells and less than 2% chance that there are more than 100 MAP CFU per litre of milk. For example, if the consumer buys raw milk at a MAP positive farm or if the farmer and his family drink that same raw milk, there is a 100% chance that they will be exposed to viable MAP in one year. But if they pasteurize the milk, there is less than 3% chance of exposure to viable MAP via milk in a year. At the dairy industry level in all three scenarios of paratuberculosis prevalence, our model shows that there is no chance of exposure to MAP through pasteurised whole milk and ultra-high temperature treated (UHT) milk. Since the chance of exposure to MAP via raw milk from positive farms is that high, we calculated that in Slovenia there is a 21–49% chance of exposure to a small amount of viable MAP when taking into account the shares of different milk on the market, an average consumption and random distribution of positive herds.

The results of our exposure assessment with respect to the underlying assumptions and uncertainties show that the risk of human exposure to MAP via milk and milk products in Slovenia is not negligible. The likelihood of exposure to MAP for the average Slovenian consumer in one year is possible, meaning that it might occur or should occur at some point. The level of MAP to which the average consumer might be exposed is low, therefore we estimated that the overall risk of exposure is low. However, it is important to emphasise that the risk of exposure to MAP via milk collected by the dairy industry and processed milk is most likely insignificant. The quantitative exposure assessment was performed for

raw milk, whole pasteurised milk and UHT milk; nevertheless, we believe that the results can be extended, at least in part, to other milk products at the respective level of production.

The main pitfalls of the conducted risk assessment are the exclusion of the import and export of raw milk, consumption milk and milk products, random selection of farms that contribute milk to a dairy plant and the assumption of the existence of an average consumer. The most important uncertainties associated with the inputs are the epidemiological features of MAP and the most important uncertainty associated with the assessment methodology is calibration and validation of the models with independent data, since the data is not yet available.

In this thesis, we showed that comprehensive insight into MAP spread and possible human exposure can be reached despite the lack of empirical data. Throughout the risk assessment process, we identified gaps where future studies should focus their efforts. Moreover, our results could provide support for informed decision-making in MAP intervention measures in Slovenia and possibly also in other countries with a similar dairy farm structure.

8 POVZETEK

Mycobacterium avium subsp. *paratuberculosis* (MAP) pri govedu povzroča paratuberkulozo ali Johnsevo bolezen, ki zmanjšuje ekonomsko učinkovitost govedoreje po vsem svetu. Predvideva se, da MAP lahko predstavlja tudi tveganje za zdravje ljudi. Potencialni viri izpostavljenosti ljudi so hrana (npr. mleko, meso), voda, okolje in neposreden stik z okuženimi živalmi. Naš cilj je bil oceniti tveganje za izpostavljenost bakteriji MAP v mleku in mlečnih izdelkih za slovenskega potrošnika.

Za doseg cilja smo uporabili prilagojen okvir analize tveganja, ki temelji na smernicah, ki so jih opredelili Svetovna zdravstvena organizacija in Organizacija za prehrano in kmetijstvo Združenih narodov (*Risk Characterization of Microbiological Hazards in Food*, 2009) ter Svetovna organizacija za zdravje živali (*Handbook on Import Risk Analysis*, 2004). Izvedli smo prvi dve komponenti analize tveganja, in sicer določitev nevarnosti in oceno tveganja, medtem ko sta upravljanje tveganj in komunikacija o tveganjih presegali namen te dizertacije.

Pri določitvi nevarnosti smo preučili značilnosti MAP, ki so pomembne za razvoj modelov in tveganje, ki ga lahko predstavlja za zdravje ljudi. MAP je majhna, zelo počasi rastoča bakterija z bogato lipidno celično steno in nagnjenjem k organizaciji celic v skupke. Celična stena je odgovorna za acidorezistenco, hidrofobnost in visoko odpornost na kemično in toplotno obdelavo. Je patogen z več gostitelji, glavni rezervoar in gostitelj pa so verjetno domači prežvekovalci. MAP lahko okuži in povzroči klinične znake pri številnih drugih domačih in divjih živalskih vrstah, vključno z nečloveškimi primati. Poleg prebavil je pri sistemske okužbi lahko prisotna tudi v mnogih drugih tkivih. Menijo, da se MAP ne more množiti zunaj gostitelja, vendar lahko v okolju preživi več kot eno leto. Okuženo govedo lahko že v subklinični fazi izloča velike količine MAP v mleku in blatu. Slednji je glavni vir za kontaminacijo okolja in širjenje patogena.

Obstaja utemeljen sum, da MAP lahko predstavlja tveganje za zdravje ljudi. Domneva se, da je povezana s Crohnovo boleznijo in nekaterimi drugimi kroničnimi boleznimi, vendar še vedno ni dokazov, ki bi potrjevali vzročnost. Kljub temu je pomembno izboljšati naše razumevanje tveganja izpostavljenosti ljudi. Žive bakterije MAP so potrdili v surovem mleku v rezervoarjih za mleko na kmetijskih gospodarstvih, v maloprodaji pa v pasteriziranem mleku, siru iz pasteriziranega in svežega mleka ter v mleku v prahu za dojenčke.

Izvedli smo kvantitativno oceno tveganja in uporabili modeliranje za premagovanje pomanjkanja empiričnih podatkov. Prav tako smo razvili razredni model za preučevanje širjenja MAP znotraj črede in model širjenja MAP na časovnem omrežju, da bi ocenili prevalenco na ravni čred v Sloveniji. Pridobljene rezultate smo uporabili v stohastičnem modelu za kvantitativno oceno izpostavljenosti.

Stohastični razredni model širjenja MAP v tipični slovenski čredi krav molznic je bil razvit v programu EMULSION (angl. *Epidemiological Multi-Level Simulation Framework*), ki temelji na uporabi končnih avtomatov. Razredi predstavljajo različna zdravstvena stanja, ki opisujejo potek bolezni. Modelirali smo dva načina poteka okužbe, pot nizkih in visokih izločevalk, pri čemer smo odrasle krave razdelili v šest razredov; enega za dovezetne krave in pet za različne stadije okužbe. Dodali smo razrede za mlade dovezetne in latentne živali, za izločene živali in za vnos novih živali v čredo. Vse vrednosti parametrov so bile deterministične, vendar so bile stopnje samodejno pretvorjene v verjetnosti, s čimer je bila

vpeljana stohastičnost. Parametri, ki opisujejo strukturo črede in demografijo, so bili ocenjeni na podlagi slovenskih podatkov iz leta 2018, ki smo jih pridobili s Kmetijskega inštituta Slovenije (KIS) in Statističnega urada Republike Slovenije (SURs). Parametri glede epidemioloških značilnosti MAP so bili pridobljeni iz objavljene literature. Nekatere vrednosti smo prilagodili na podlagi strokovnega mnenja in kalibracije modela, katere namen je bil zagotoviti stabilnost velikosti črede. Okužba se je začela z vnosom ene okužene živali v čredo, prosto paratuberkuloze. Širjenje MAP znotraj črede smo z dnevnim časovnim korakom simulirali 10 let in s 1.000 ponovitvami. Izvedli smo notranjo validacijo modela in analizo občutljivosti za epidemiološko najpomembnejše parametre.

V nasprotju z našimi pričakovanji model ni pokazal večje razširjenosti MAP znotraj črede, kot je bilo predhodno ocenjeno. Čeprav se je povprečna velikost kmetijskih gospodarstev v Sloveniji v zadnjih letih povečala (z 12,5 krav molznic v letu 2008 na 17,1 kravo molznic na kmetijo v letu 2018), to ni zagotovilo boljših pogojev za širjenje MAP. Ker smo razredni model razvili za zagotovitev podatkov za model ocene izpostavljenosti, smo prevalenco ter delež subklinično in klinično okuženih krav izračunali samo na podlagi števila odraslih živali. Rezultati so pokazali, da je povprečna prevalenca znotraj črede 7,13 % s standardnim odklonom (SD) 8,43 %, mediano 5,56 % in najvišjo prevalenco 70,59 %. V povprečju je bilo v čredi 0,47 % klinično okuženih živali (SD 1,63 %) in 4 % subklinično okuženih živali (SD 5,42 %), pri čemer je bila mediana v obeh primerih 0 %. Naši rezultati kažejo, da je za proučevanje širjenja MAP v čredah različnih velikosti potrebno uporabiti različne modele. Kolikor nam je znano, smo razvili prvi stohastični razredni model, ki je zasnovan posebej za majhno čredo krav molznic. Predstavljeni model je primeren tudi za druge države s podobno strukturo mlečnih gospodarstev, saj lahko služi tudi za preizkušanje učinkovitosti različnih kontrolnih ukrepov.

Z analizo omrežja smo preučili strukturo premikov goveda v Sloveniji in ovrednotili potencial za širjenje MAP na časovnem omrežju. Podatke o premikih govedi smo pridobili z Uprave Republike Slovenije za varno hrano, veterinarstvo in varstvo rastlin. V študijo smo vključili premike od avgusta 2011 do julija 2016. Ker so nas zanimali premiki, ki so epidemiološko pomembni za širjenje bolezni, smo izločili vse premike na gospodarstva, ki so ustrezala končnim vozliščem oziroma krajiščem. Statično analizo omrežja smo izvedli na mesečnih in letnih posnetkih omrežja z uporabo programske opreme Pajek, ciljno usmerjeno odstranjevanje vozlišč pa z uporabo R paketa igraph. Poleg tega smo s pomočjo programskega jezika Python simulirali širjenje MAP na časovnem omrežju s tako imenovanim SI modelom (angl. *susceptible-infectious*; dovzetin-inficiran).

Kljub temu da so bile mere središčnosti in pomembnosti med leti primerljive, pa so se povezave med gospodarstvi iz leta v leto nekoliko spreminjale. Pomembnost posameznega gospodarstva za povezanost omrežja je bila zelo heterogena. Najvplivnejša gospodarstva v omrežju so bili zbirni centri, planinski pašniki in pašniki. Pri razčlenjevanju omrežja je bilo ciljano odstranjevanje vozlišč, razvrščenih glede na vrednosti mer pomembnosti in središčnosti iz prejšnjih let, bistveno učinkovitejše kot naključno odstranjevanje vozlišč. SI model je temeljil na empirično izračunani prevalenci paratuberkuloze v Sloveniji in štirih scenarijih verjetnosti prenosa MAP z vrednostmi 0,02, 0,15, 0,25 in 0,40. S 1.000 ponovitvami modela za vsako navedeno verjetnost prenosa je bila ob koncu proučevanega obdobja napovedana prevalenca na ravni čred: 21,30 %, 38,89 %, 49,21 % in 59,85 %.

V disertaciji smo predstavili prvo poglobljeno analizo kompleksnega omrežja premikov goveda v Sloveniji. Uporabljeni pristop modeliranja širjenja MAP med čredami z razredčenimi omrežji se prav

tako lahko uporabi za modeliranje drugih poti prenosa bolezni med čredami in tudi drugih bolezni. Naši rezultati kažejo, da bi analiza omrežja premikov govedih lahko pripomogla k optimizaciji spremljanja in obvladovanja paratuberkuloze v Sloveniji.

Za oceno potencialne izpostavljenosti slovenskega potrošnika bakteriji MAP preko mleka in mlečnih izdelkov smo s programom @RISK razvili stohastični model za kvantitativno oceno tveganja s simulacijami Monte Carlo. Kontaminacijo surovega in pasteriziranega polnomastnega mleka ter možno izpostavljenost ljudi smo modelirali na ravni kmetije in na ravni mlečne industrije. V modelu smo uporabili podatke pridobljene s SURS-a, KIS-a, Agencije RS za kmetijske trge in razvoj podeželja, Gospodarske zbornice Slovenije in iz literature ter preko strokovnega mnenja. Vsi podatki, pridobljeni za Slovenijo, so se nanašali na leto 2018. Podatkom o demografiji in proizvodnji na ravni kmetije, pa tudi rezultatom prejšnjih dveh modelov, smo prilagodili verjetnostne porazdelitve, ki smo jih izbrali na podlagi najnižje vrednosti Akaike informacijskega merila. Verjetnostne porazdelitve o zmanjšani prireji mleka, kontaminaciji z MAP in učinkovitosti mlečnega filtra, smo ocenili na podlagi literature in strokovnega mnenja. Če je model simuliral majhno čredo, smo delež klinično in subklinično okuženih krav v čredi izračunali na podlagi rezultatov našega razrednega modela, v nasprotnem primeru pa smo uporabili porazdelitev na podlagi objavljenih podatkov. Mejna vrednost za majhno čredo, ki smo jo postavili na podlagi mnenja strokovnjaka, je bila 50 krav molznic. Zbiranje mleka v mlekarnah smo modelirali s 100 modeli na ravni kmetijskega gospodarstva in treh scenarijev prevalence MAP na ravni čred, ki so temeljili na modelu širjenja MAP na časovnem omrežju z izključenim najslabšim scenarijem. S pomočjo metode vzorčenja z latinskimi hiperkockami smo izvedli 10.000 ponovitev simulacij Monte Carlo. Poleg tega smo opravili analizo občutljivosti in preizkusili različne mejne vrednosti za majhno čredo.

Naši rezultati kažejo, da je v primeru kmetije, okužene z MAP, verjetnost, da je surovo mleko v rezervoarju za mleko in s tem tudi surovo konzumno mleko na ravni kmetije kontaminirano z MAP, visoka. Izračunali smo, da je možnost, da liter mleka vsebuje celice MAP, višja od 85 %, in nekoliko manj kot 2 %, da vsebuje več kot 100 CFU MAP na liter mleka. Na primer, če potrošnik kupuje surovo mleko na kmetiji, kjer je prisotna paratuberkuloza, ali pa kmet in njegova družina to mleko pijejo, obstaja 100-odstotna verjetnost, da bodo v enem letu izpostavljeni živim celicam MAP. V primeru, da bi mleko pasterizirali, se možnost izpostavljenosti MAP preko mleka v enem letu zniža na manj kot 3 %. V vseh treh scenarijih prevalence MAP na ravni mlečne industrije je bil rezultat simulacije, da verjetnost za izpostavljenost MAP preko pasteriziranega polnomastnega mleka in preko mleka, obdelanega pri ultra visoki temperaturi ne obstaja. Glede na delež posameznih vrst mleka na trgu in ob predpostavkah, da povprečni potrošnik uživa vse omenjene vrste mleka in da je porazdelitev pozitivnih čred naključna, je možnost izpostavljenosti nizki količini živih celic MAP v Sloveniji od 21 do 49 %.

Rezultati kvantitativne ocene izpostavljenosti z upoštevanjem osnovnih predpostavk in negotovosti kažejo, da tveganje za izpostavljenost ljudi bakteriji MAP preko mleka in mlečnih izdelkov v Sloveniji ni zanemarljivo. Za povprečnega slovenskega potrošnika je verjetnost izpostavljenosti MAP v enem letu možna, kar pomeni, da se lahko zgodi ali bi se moralo zgoditi v nekem trenutku. Količina MAP, ki bi ji lahko bil izpostavljen povprečni potrošnik, pa je nizka, zato smo ocenili, da je splošno tveganje za izpostavljenost nizko. Pomembno je poudariti, da je tveganje za izpostavljenost MAP preko mleka, ki ga zbirajo mlekarnice, in preko predelanega mleka, najverjetneje zanemarljivo. Čeprav smo kvantitativno oceno izpostavljenosti izvedli le za surovo mleko, pasterizirano polnomastno mleko in

mleko, obdelano pri ultra visoki temperaturi, menimo, da se rezultati lahko do neke mere prenesejo tudi na druge mlečne izdelke na zadevni ravni proizvodnje.

Glavne pomanjkljivosti predstavljene ocene tveganja so izključitev uvoza in izvoza surovega mleka, mleka in mlečnih izdelkov v maloprodaji, naključna izbira kmetij, ki oddajo mleko v mlekarno, in predpostavka o obstoju povprečnega potrošnika. Najpomembnejše negotovosti, povezane s spremenljivkami v modelu, so epidemiološke značilnosti MAP. Najpomembnejša negotovost, povezana z metodologijo, je kalibracija in validacija modela z neodvisnimi podatki, saj podatki še niso na voljo. Naši rezultati bi lahko kljub omenjenim pomanjkljivostim nudili podporo za informirano odločanje o intervencijskih ukrepih za nadzor MAP v Sloveniji.

V tej disertaciji smo pokazali, da je kljub pomanjkanju empiričnih podatkov mogoče pridobiti celovit vpogled v širjenje MAP in možnost izpostavljenosti ljudi. Skozi postopek ocene tveganja smo prepoznali vrzeli, kamor bi bilo v prihodnjih študijah smiselno usmeriti nadaljnja prizadevanja. Poleg tega so naši rezultati lahko uporabni tudi za druge države, ki imajo podobno strukturo mlečnih gospodarstev.

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