



## Control Tools

### Diagnostics availability

#### Commercial diagnostic kits available worldwide

Environmental mastitis (E. coli, Str. uberis)

Routine laboratory diagnostics are largely based on bacteriological culture or on PCR, with different EU countries favouring different testing systems.

In addition to species identification, antimicrobial susceptibility testing is offered by some laboratories. This can be limited to routine differentiation of penicillin susceptible and penicillin resistant *S. aureus* (phenotypically or genotypically) or cover a broad range of compounds and pathogens.

Increasingly, matrix-assisted laser desorption ionisation time of flight (MALDI-ToF) analysis is used for species identification after initial bacteriological culture.

For on-farm culture, a growing array of tests is available, including but not limited to Petrifilm based culture and use of selective indicator media. Some on-farm tests include antimicrobial susceptibility testing. Tests are largely based on culture and take up to 24 hrs. Some tests only differentiate gram-positive versus gram-negative species whereas other tests provide additional detail at genus or species level.

GAPS:

Rapid on-farm detection (within 8 hrs).

Rapid antimicrobial susceptibility testing (on farm or off farm)

Culture-free diagnostics to overcome concerns about on-farm propagation of potential biohazards.

## Commercial diagnostic kits available in Europe

Environmental mastitis (E. coli, Str. uberis)

See Section “Commercial diagnostic kits available worldwide”.

## Diagnostic kits validated by International, European or National Standards

Environmental mastitis (E. coli, Str. uberis)

No official national or international standards exist for detection of mastitis so validation based on such standards is not possible. Some on-farm tests claim on their web sites to be recognized as the Gold Standard but there is no scientific or regulatory evidence to support such claims.

## Diagnostic method(s) described by International, European or National standards

Environmental mastitis (E. coli, Str. uberis)

None.

GAP: Diagnostic method(s) described by International standards (i.e. OIE / EU).

## Commercial potential for diagnostic kits in Europe

Environmental mastitis (E. coli, Str. uberis)

Yes. In addition to laboratory-based diagnostics, on-farm diagnostics are increasingly being explored or market by animal health companies.

GAP:

Accurate cost/benefit assessment of new methods, including knowledge of socio-economic, cultural and behavioural drives of uptake of control measures.

## DIVA tests required and/or available

Environmental mastitis (E. coli, Str. uberis)

Not applicable because vaccination does not interfere with diagnostic methods.

## Opportunities for new developments

Environmental mastitis (E. coli, Str. uberis)

On-farm diagnostics and algorithms to support treatment decision making.

Diagnostics at subspecies (strain) level, particularly if relevant as predictor for cure of affected animal or transmission to other animals.

Microbiome based diagnostics.

GAP:

Socio-economic, behavioural and cultural drivers of farm-based diagnostics; technical limitations to testing, particularly turn-around-time for culture-based diagnostics or spectrum of targets for molecular diagnostics.

Knowledge of prognostic markers.

Technical feasibility and affordability; knowledge of healthy microbiome and ways to influence microbiome.

## Vaccines availability

### Commercial vaccines availability (globally)

Environmental mastitis (E. coli, Str. uberis)

J5 bacterins, formulated with a mutant strain of Escherichia coli O111:B4 (Rc mutant) lacking the “O” antigen capsule of the cell wall but with the core lipopolysaccharide (endotoxin) intact form the basis for E. coli mastitis vaccines.

The genetically-engineered Gram-negative bacterin, Re-17, comprised of the universal core antigen common to all Gram-negative bacteria, as present in Endovac-Dairy is claimed to protect against E. coli mastitis and other endotoxin-mediated diseases caused by E. coli, Salmonella, Pasteurella multocida, and Pasteurella (Mannheimia) haemolytica.

StartVac/TopVac (inactivated Escherichia coli (strain J5) and inactivated Staphylococcus aureus (CP8, strain SP 140) (variable launch dates).

Lysigin (lysed culture of highly antigenic polyvalent somatic antigen containing phage types I, II, III, IV and miscellaneous groups of Staph. aureus).

Evidence of efficacy and economic viability is mixed, and generally stronger for E. coli vaccines than for S. aureus vaccines.

In several countries, use of autogenous mastitis vaccines is permitted. Such vaccines include, but are not limited to vaccines against Mycoplasma, Klebsiella and Strep. uberis. Efficacy is generally unproven, and evidence of product safety may be the only evidence required to obtain authorisation for use.

UBAC Subunit vaccine against mastitis caused by Streptococcus uberis (launched 2018).

KLEBVax SRP vaccine using siderophore receptor and porin technology against mastitis caused by Klebsiella (launched 2018).

All vaccines listed above are for dairy cattle. For sheep and goats, an inactivated *Staph. aureus* vaccine (VIMCO) is available to prevent subclinical mastitis.

Note: this list may not cover all vaccines available beyond Europe and North America.

#### GAPS:

Standardized methods to evaluate effectiveness of the vaccines under field conditions.

Independent scientific evidence for efficacy of *S. aureus* vaccines under field conditions.

Availability of vaccines to protect from mastitis caused by *Streptococcus agalactiae*, *Streptococcus dysgalactiae*, *Klebsiella* spp. or *Mycoplasma* spp.

### Commercial vaccines authorised in Europe

Environmental mastitis (*E. coli*, *Str. uberis*)

StartVac/TopVac (inactivated *Escherichia coli* (strain J5) and inactivated *Staphylococcus aureus* (CP8, strain SP 140) (launched 2010).

UBAC Subunit vaccine against mastitis caused by *Streptococcus uberis* (launched 2018).

This list is up to date at the time of submission of this document but vaccine availability may change over time. Mention of a product does not constitute an endorsement.

#### GAPS:

Standardized methods to evaluate effectiveness of the vaccines under field conditions.

### Marker vaccines available worldwide

Environmental mastitis (*E. coli*, *Str. uberis*)

Not applicable.

GAP:

None

## Marker vaccines authorised in Europe

Environmental mastitis (E. coli, Str. uberis)

Not applicable.

## Effectiveness of vaccines / Main shortcomings of current vaccines

Environmental mastitis (E. coli, Str. uberis)

The mammary gland is an immunoprivileged body site, implying that its immunology is largely different from other body sites and that vaccine development is biologically challenging. This is compounded by the fact that environmental mastitis is a disease complex with a variety of causative agents, which would need to be covered by multiple vaccines or multi-valent vaccines.

For most mastitis vaccines, repeated administration is needed to maintain a protective immune response. This limits the practicality and commercial viability of vaccines. Improved vaccine efficacy after single administration is desirable.

Independent scientific evidence of vaccine effectiveness is available for E. coli vaccines (reduced milk yield losses, reduce risk of mortality, cost-effective), albeit it with variable efficacy and cost-effectiveness at the level of individual farms. Similar evidence is weak for S. aureus vaccines and not available yet for S. uberis vaccines.

GAPS:

Knowledge of the protective immune response to environmental mastitis pathogens.

Knowledge of ways to enhance the protective immune response to environmental mastitis pathogens.

Independent scientific evidence for efficacy of *S. aureus* or *S. uberis* vaccines under field conditions.

Multivalent vaccines.

Needle free and/or automated administration techniques.

## Commercial potential for vaccines in Europe

Environmental mastitis (*E. coli*, *Str. uberis*)

Considering the pressure to reduce antimicrobial use as a tool to control mastitis and the pressure on availability of skilled farm labour, mastitis vaccines are increasingly likely to be a commercially viable control tool for mastitis.

GAP:

Efficacious, and cost-effective vaccines that would not require multiple administrations to provide protection.

## Regulatory and/or policy challenges to approval

Environmental mastitis (*E. coli*, *Str. uberis*)

Currently none.

## Commercial feasibility (e.g manufacturing)

Environmental mastitis (E. coli, Str. uberis)

Two mastitis vaccines have become commercially available in Europe in the past 10 years for dairy cattle, and one for sheep and goats.

## Opportunity for barrier protection

Environmental mastitis (E. coli, Str. uberis)

Not applicable.

## Opportunity for new developments

Environmental mastitis (E. coli, Str. uberis)

The role of non specific udder immune defences has been suggested to be very important in preventing new infections. Very few studies addressed these aspects.

GAP:

Methods to improve the non specific immune defences are needed (immunomodulators ?) which enable the udder to reduce its susceptibility, independently from the bacteria involved.



## Pharmaceutical availability

### Current therapy (curative and preventive)

Environmental mastitis (E. coli, Str. uberis)

Current curative therapy is based on use of antimicrobials (intramammary and/or systemic), possibly supplemented with NSAIDs and supportive treatment (see Section “Main means of prevention, detection and control>Therapeutics).

In the dry period, antimicrobials are also used to prevent mastitis. Such use is likely to become restricted. Non-antimicrobial alternatives for prevention exist in the form of teat sealants (see Section “Main means of prevention, detection and control.> mechanical and biological control”).

GAP:

Need to educate veterinarians and farmers regarding restrictions on use of Highest Priority Critically Important Antimicrobials in animal agriculture and availability of alternatives protocols (e.g. culture-based treatment) and tools (e.g. teat sealants).

### Future therapy

Environmental mastitis (E. coli, Str. uberis)

Due to societal pressure to reduce the use of antimicrobials in animal agriculture, the availability of antimicrobial products for treatment of bovine mastitis can be expected to decrease. This is particularly true for WHO-defined Highest Priority Critically Important Antimicrobials such as 3<sup>rd</sup>/4<sup>th</sup> Generation Cephalosporins, fluoroquinolones and macrolides, all of which are currently still used for mastitis treatment in several European and non-European countries.

Future therapy may be based on other principles, e.g. microbiome manipulation, phage therapy or antimicrobial peptides. With the exception of the latter, there is no evidence yet that such treatments are biologically and economically feasible.

GAPS:

Understanding of the teat and gland microbiome and methods to influence its composition.

Phage therapy.

Antimicrobial peptides.

New antimicrobial compounds, e.g. derived from algae.

## Commercial potential for pharmaceuticals in Europe

Environmental mastitis (E. coli, Str. uberis)

Limited based on investment made by pharmaceutical companies, despite importance from the perspective of the farming industry.

GAP:

Lack of financial incentive for pharmaceutical companies to invest in product development.

## Regulatory and/or policy challenges to approval

Environmental mastitis (E. coli, Str. uberis)

Product registration is a time consuming and expensive process.

## Commercial feasibility (e.g manufacturing)

Environmental mastitis (E. coli, Str. uberis)

See Section “Commercial potential for pharmaceuticals in Europe”.

## Opportunities for new developments

Environmental mastitis (E. coli, Str. uberis)

See Section “Future therapy”.

## New developments for diagnostic tests

### Requirements for diagnostics development

Environmental mastitis (E. coli, Str. uberis)

The development of rapid identification and sensitivity testing of mastitis-causing pathogens may be beneficial, particularly if testing can be used to inform treatment decisions and reduce use of antimicrobials without compromising dairy cattle welfare.

GAPS:

Knowledge of socio-economic, behavioural and cultural drivers of farm-based diagnostics.

Technical improvements, notably turn-around time, ease of use and cost.

Integration with host-data to inform decision making.

Combined pathogen detection and susceptibility testing

## Time to develop new or improved diagnostics

Environmental mastitis (E. coli, Str. uberis)

Several methods have recently come to market or are currently under development with commercial or research funding.

## Cost of developing new or improved diagnostics and their validation

Environmental mastitis (E. coli, Str. uberis)

Perceived to be justified based on recent investment in diagnostic test development. Societal pressure to reduce antimicrobial use is perceived as a driver for on-farm diagnostics.

## Research requirements for new or improved diagnostics

Environmental mastitis (E. coli, Str. uberis)

Engineering expertise to simplify and ruggedize diagnostic tools or equipment.

Optics to speed up detection of pathogens or susceptibility patterns (similar developments in human medical microbiology allow such detection within hours, but currently at very high cost).

Reductions in cost and complexity of data generation and analysis for microbiome data.

Improved methods to differentiate bacterial contamination of samples from infection, e.g. through improved sample collection methods or combination with inflammatory markers.

Socio-economic expertise to understand requirements for uptake.

GAP:

See Information available, which lists requirements or gaps.

## Technology to determine virus freedom in animals

Environmental mastitis (E. coli, Str. uberis)

Not applicable.

## New developments for vaccines

### Requirements for vaccines development / main characteristics for improved vaccines

Environmental mastitis (E. coli, Str. uberis)

See Section "Vaccines availability >Effectiveness of vaccines/main shortcomings of current vaccines".

### Time to develop new or improved vaccines

Environmental mastitis (E. coli, Str. uberis)

Commercial timelines indicate that it takes ca. 10 years to progress from target identification to commercial availability.

GAP:

Advances are needed in understanding of the host immune response, vaccine production and administration methods, and socio-economic drivers of vaccine uptake.

## Cost of developing new or improved vaccines and their validation

Environmental mastitis (E. coli, Str. uberis)

Development costs are significant and estimated to exceed US\$15-20M per pathogen.

## Research requirements for new or improved vaccines

Environmental mastitis (E. coli, Str. uberis)

Multi-disciplinary, multi-site approach with interaction between commercial and academic partners, including biological and social scientists.

GAP:

Funding for multi-disciplinary collaborations around vaccine development and uptake.

## New developments for pharmaceuticals

## Requirements for pharmaceuticals development

Environmental mastitis (E. coli, Str. uberis)

See Section “Pharmaceutical availability > Future therapy”.

GAP:

Lack of economic incentive for pharmaceutical companies to invest in animal health products.

## Time to develop new or improved pharmaceuticals

Environmental mastitis (E. coli, Str. uberis)

Could be significant.

GAP:

Lack of economic incentive for pharmaceutical companies to invest in animal health products.

## Cost of developing new or improved pharmaceuticals and their validation

Environmental mastitis (E. coli, Str. uberis)

Could be significant.

GAP:

Lack of economic incentive for pharmaceutical companies to invest in animal health products.

## Research requirements for new or improved pharmaceuticals

Environmental mastitis (E. coli, Str. uberis)

Multi-disciplinary, multi-site approach with interaction between commercial and academic partners, including biological and social scientists

GAP:

Funding for multi-disciplinary collaborations around vaccine development and uptake.

## Disease details

### Description and characteristics

### Pathogen

Environmental mastitis (E. coli, Str. uberis)

Environmental mastitis is defined based on epidemiological criteria (environment as main source of the pathogen with transmission from the environment to individual cows) and contrasted to contagious mastitis (infected cow as main source of the pathogen with transmission from cow to cow), which is a key distinction in terms of prevention and control measures. Many mastitis pathogens can originate from or be spread via the environment. They include gram-negative coliform species (*Escherichia coli*, *Klebsiella* spp., *Enterobacter* spp.), gram-negative non-coliform species (e.g. *Serratia* spp., *Pseudomonas* spp.), gram-positive catalase negative species (*Streptococcus* spp., *Enterococcus* spp., *Lactococcus* spp.), gram-positive catalase positive species (*Staphylococcus aureus* and non-aureus staphylococci) and non-bacterial species (e.g. yeast, *Prototheca* spp.). Among the major mastitis pathogens, i.e. those



with the highest prevalence or incidence, and the strongest impact on udder health and milk quality, *E. coli* is most likely to be environmental, followed closely by *Klebsiella*, and at some distance by *Streptococcus uberis* and *Streptococcus dysgalactiae*, and *Staph. aureus*. *Staph. aureus* is included in the environmental mastitis analysis because *Staph. aureus* can be an environmental pathogen.

GAP:

Standardised typing systems at subspecies (strain) level to establish a likely environmental origin or mode of transmission or to predict virulence.

## Variability of the disease

Environmental mastitis (*E. coli*, *Str. uberis*)

Within the genera and species mentioned above, there is considerable genetic heterogeneity at species and subspecies level. In addition, likely sources and transmission routes differ between species. For example. Coliform species and gram-positive catalase-negative species are found in faeces and any substrate that is contaminated with faeces, staphylococcal species are often associated with mammalian skin, and *Pseudomonas* and *Proteus* are often associated with water. Environmental mastitis is a heterogeneous disease syndrome rather than a single disease caused by a single organism.

GAPS:

Pathogen populations evolve continuously in response to selective pressures, and new patterns of infection emerge. For example, *Klebsiella* was long thought to be an environmental pathogen but potential contagious transmission has been documented. Conversely, *Streptococcus agalactiae* was long considered to be a contagious pathogen, but potential environmental sources and transmission routes (faeces, humans) have recently been recognized. Little is known about mechanisms of host- or niche adaptation.

Routine culture based phenotypic diagnostics may fail to identify bacterial genera or species correctly, e.g. *Klebsiella* spp. may be mistaken for *E. coli* and *Lactococcus* spp. or *Enterococcus* spp. may be mistaken for *Streptococcus* spp. Routine culture based phenotypic diagnostics lack the ability to reliably identify coagulase negative *Staphylococcus* spp.

Many of the subtleties in 1.1 and 1.2 are well known from research papers and outbreak investigations, but there is a gap in uptake of this knowledge in veterinary practice and herd health management programs.

## Stability of the agent/pathogen in the environment

## Environmental mastitis (E. coli, Str. uberis)

Environmental mastitis pathogens may persist for weeks (Strep. uberis) or months (E. coli) in the environment. Waterborne mastitis pathogens (e.g. Pseudomonas) may persist for weeks in biofilms, e.g. in the milking equipment.

### GAP:

There is limited knowledge about the relative contribution of introduction vs. persistence or amplification in(to) the environment to the prevalence of environmental pathogens. For environmental pathogens of faecal or (muco)cutaneous origin, presence of cows is likely to provide an ongoing source of introduction of pathogens into the environment but the contribution of ongoing shedding vs. growth in the environment has not been quantified.

## Species involved

### Animal infected/carrier/disease

## Environmental mastitis (E. coli, Str. uberis)

Dairy cattle are the main focus of this disease and product analysis. Most bovine mastitis pathogens may also affect water buffalo, whilst some also affect goats (notably Staphylococcal species including Staph. aureus) or sheep (notably Staph. aureus, Strep. uberis and Strep. dysgalactiae). In the context of a European analysis, camels will not be considered here. Ovine mastitis may also be caused by Mannheimia haemolytica, which is not found in cattle, whereas one of the most common causes of environmental mastitis in cattle, i.e. E. coli, is rarely found in sheep.

People, pigs and poultry may be a source of Staph. aureus, including methicillin resistant Staph. aureus (MRSA) and, possibly, extended spectrum betalactamase (ESBL) resistant E. coli. Dogs and cats may be a source of Streptococcus canis. For S. aureus and S. canis, initial introduction from an environmental source (non-bovine host species) may be followed by contagious transmission.

### GAP:

The biological basis of host adaptation of some pathogen species to specific host species (e.g. E. coli in cattle vs. Mannheimia in sheep) is poorly understood.

## Human infected/disease

Environmental mastitis (E. coli, Str. uberis)

Staphylococcal carriage and/or infection may result in transmission between people and cattle, whereby transmission appears to be possible in either direction. This is particularly true for Staph. aureus, including MRSA, whilst Staph. epidermidis is suggested to be transmitted from humans to cattle more than from cattle to humans.

In recent years, the possibility of interspecies transmission of Streptococcus agalactiae between cattle and people has been recognized in countries that strive to eradicate this pathogen from the national dairy herd. In particular, sequence type (ST)196, which is recognized as an emerging pathogen in humans, is a common strain among dairy herds in some countries.

GAP:

Frequency of transmission of pathogens from cattle to humans and from humans to cattle.

## Vector cyclical/non-cyclical

Environmental mastitis (E. coli, Str. uberis)

Insects (e.g. horn flies, wasps) may contribute to transmission of pathogens (e.g. Staph. aureus, Strep. dysgalactiae, Peptostreptococcus indolicus /summer mastitis). Insect-based transmission originates with infected cows as the primary source of pathogens. Therefore, this will be considered contagious rather than environmental transmission, i.e. it falls out with the scope of this analysis.

## Reservoir (animal, environment)

Environmental mastitis (E. coli, Str. uberis)

The ruminant gastro-intestinal tract (coliforms, gram-positive catalase negative cocci) and skin (gram-positive catalase positive cocci) are important reservoirs of environmental pathogens. Water may be a reservoir of some environmental pathogens (*Pseudomonas*, *Proteus*, *Prototheca*). Unused bedding material can be a source of pathogens (e.g. *Klebsiella* in green wood shavings). Once bedding is in use, i.e. in contact with cattle and cattle faeces, it becomes an important reservoir of environmental mastitis pathogens.

Unusual reservoirs have been documented in outbreaks and include animal health products such as teat wipes (*Pseudomonas*) and chlorhexidine teat dip (*Serratia*).

#### GAPS:

Diagnostic tools for monitoring of pathogen load in environmental sources, including but not limited to new bedding material, used bedding, water, and animal health care products such as teat dips or wipes.

There is a gap in knowledge regarding the relationship between nutrition and pathogen shedding, although it has been suggested that starch-rich diets may contribute to excretion of *Klebsiella* whereas pasture-based diets may contribute to excretion of *S. uberis*.

The impact of bedding type and bedding management on environmental pathogen loads is poorly understood.

The role of drinking water in faecal-oral transmission routes is poorly quantified.

## Description of infection & disease in natural hosts

### Transmissibility

Environmental mastitis (*E. coli*, *Str. uberis*)

The defining characteristics of environmental mastitis is that cows become infected from environmental sources, rather than from the infected mammary gland of other cattle in the herd.

The risk of environmental mastitis depends on the balance between pathogen load from the environment and host resistance/resilience to infection (i.e. the ability to prevent or cope with infection).

For some pathogen species (*Strep. agalactiae*, *E. coli*, *Klebsiella pneumoniae*) the ability to ferment lactose is associated with an increased ability to cause mastitis, based on higher prevalence of lactose operons in bovine milk derived isolates compared to isolates from other (environmental, incl. faecal and human) sources.

GAP:

There is no knowledge of the impact of nutrition on faecal shedding of environmental mastitis pathogens.

There is limited knowledge of host genetic factors associated with host resistance or resilience to environmental mastitis caused by different pathogens.

With the exception of the role of negative energy balance and vitamin E/selenium in susceptibility to coliform mastitis, there is limited knowledge of the impact of nutrition on host resistance and resilience to environmental mastitis.

Molecular markers of the ability of environmental organisms to cause mastitis (pathogenicity, virulence) are largely unknown for all environmental mastitis pathogens.

## Pathogenic life cycle stages

Environmental mastitis (*E. coli*, *Str. uberis*)

Some environmental pathogens (e.g. *Pseudomonas*, *Strep. agalactiae*) may have the ability to survive in biofilm.

GAP :

No knowledge of role of environmental biofilms, e.g. in milking equipment or drinking water, in causation of mastitis.

## Signs/Morbidity

## Environmental mastitis (*E. coli*, *Str. uberis*)

Mastitis can be differentiated into subclinical mastitis, i.e. inflammation of the mammary gland without any overt signs of disease, and clinical mastitis, i.e. inflammation of the mammary gland with overt signs of disease.

Clinical mastitis is commonly differentiated into grades based upon severity. Multiple scoring methods for severity have been proposed. At its most basic, three categories can be identified, i.e.

- Mild = abnormalities in milk (e.g. clots, flakes, discoloration) but not in udder or general health;
- Moderate = abnormalities in milk and udder (e.g. hot, swollen, painful, discoloration) but not in general health;
- Severe = abnormalities in milk, udder and general health (e.g. fever, depression, anorexia, dehydration).

Subclinical mastitis is more commonly associated with gram-positive than with gram-negative mastitis, although chronic subclinical mastitis due to coliform pathogens may occur. Conversely, severe clinical mastitis is more commonly associated with gram-negative than with gram-positive mastitis, although acute severe mastitis due to e.g. *Staph. aureus* may occur.

Classification of severity and causative agent is important to inform treatment decisions. For severe cases, treatment targets include infection (antimicrobial agents), inflammation (anti-inflammatory agents), and supportive therapy (hydration, minerals). In severe mastitis cases, sepsis can occur. Antimicrobial treatment may target intramammary infection as well as sepsis.

For non-severe mastitis, there is growing interest in selective use of antimicrobial therapy in response to economic and societal pressures. The rationale behind this approach is that non-severe mastitis caused by gram-positive pathogens is more likely to cure with antimicrobial therapy than without, whereas antimicrobial therapy is not indicated for culture negative mastitis, nor for mastitis caused by gram-negative pathogens, yeasts or algae. For gram-negative mastitis, anti-inflammatory treatment is recommended.

Morbidity (the proportion of animals in the herd that is affected) is highly variable between herds, groups within herds (e.g. fresh cows vs. mid-lactation), seasons (country-specific impact of heat/housing/humidity), farm types (e.g. free stall vs. tie-stall; open vs. closed floor), etc. Outbreaks, by definition, are short periods of unusually high morbidity, whereby more than 10% of a herd may be affected in a few weeks to months.

GAP:

Fast, accurate and practical tools to determine the causative agent of mastitis and inform decision making around treatment and culling are needed. This includes identification at pathogen level (notably gram-positive vs. gram-negative or culture negative) as well as detection of prognostic indicators (probability of cure, impact on milk production or longevity). Important features of tests for immediate decision making include low cost, limited hands-on time, rapid turn-around, high predictive value, low biological risk to humans, ease of use, and easy of decision making. In addition, high sensitivity and specificity are necessary.

Potential of precision dairy farming: automatic and early detection of cows with behavioural or clinical signs of mastitis to facilitate immediate intervention and reduce production losses, disease severity and mortality. Highly relevant for animal welfare, particularly between milkings for cows that are milked 2x/day or less (which is not unusual in automated milking systems).

## Incubation period

Environmental mastitis (E. coli, Str. uberis)

Incubation time of clinical disease varies by pathogen species and strain, with experimentally induced E. coli mastitis causing clinical signs within 12 hours and experimentally induced Strep. uberis mastitis including clinical signs up to 3 days after challenge. Environmental mastitis pathogens may cause subclinical infections and for such infections the definition of incubation time (immediately after infection? Undefined or none because clinical signs do not occur?) is a matter of semantics. Coliform infections that occur during the dry period, particularly for E. coli, may not result in clinical signs until days or weeks after parturition.

Based on farmers' experience, incubation time can be less than 8 hrs, i.e. the time between two milkings.

GAPS:

Diagnostic tests that can predict the onset of severe clinical mastitis. This could be based on a combination of information from e.g. the milking machine, biomarkers and behavioural indicators (lying time, rumination) with associated data mining (precision farming).

Prognostic tests to predict whether a subclinical infection, e.g. as present at calving, is likely to resolve spontaneously or to result in clinical mastitis.

## Mortality

## Environmental mastitis (E. coli, Str. uberis)

Highly variable in cattle, e.g. high for environmental S. aureus mastitis around parturition and for severe E. coli or Klebsiella mastitis but low for mild to moderate clinical mastitis. In sheep, S. aureus and Mannheimia may cause high mortality.

Mortality estimates depend on whether culling due to chronic mastitis is included. For example, cows with chronic and therapy non-responsive mastitis due to Strep. uberis or Klebsiella may be culled because of high SCC or low yield.

## GAPs :

Therapeutic agents (antimicrobial or otherwise) for treatment of severe clinical mastitis, particularly as caused by S. aureus or Klebsiella in dairy cattle.

Prognostic indicators for cure.

## Shedding kinetic patterns

### Environmental mastitis (E. coli, Str. uberis)

Highly variable across species and strains, ranging from transient (several days) and high levels of shedding to chronic (months) and high levels of shedding to chronic but intermittent shedding.

Historically, E. coli was considered to cause transient infections with clinical signs and high shedding but chronic E. coli infection with intermittent shedding and periodic clinical manifestation is now recognized. Conversely, Strep. agalactiae was historically considered to cause chronic subclinical infection but transient clinical S. agalactiae mastitis due to human-associated strains has now also been described.

Many other pathogen species, including Klebsiella and S. uberis, can also have variable levels and durations of shedding, ranging from intermittent to high shedding and from transient to chronic shedding.

## GAP:

It is unknown whether shedding patterns are associated with probability of cure or future milk production.



## Mechanism of pathogenicity

Environmental mastitis (*E. coli*, *Str. uberis*)

Highly variable across species and strains. For gram-negative pathogens, lipopolysaccharide induced inflammation may have more clinical impact than infection, with potential aggravation by anti-infective treatment.

Role of bacterial adhesion to and invasion into mammary epithelium is a topic of debate, both for coliforms and for gram-positive pathogens.

Efficacy of host immune response and mechanisms of resistance to host immune response also differs between genera, species and strains.

GAPS:

Host immune response to infection, particularly role of cells other than polymorphonuclear leucocytes, e.g. lymphocytes or macrophages.

Mechanisms of immune evasion, including biofilm formation, invasion and intracellular survival in host (epithelial) cells in vivo (hitherto only documented in vitro).

## Zoonotic potential

### Reported incidence in humans

Environmental mastitis (*E. coli*, *Str. uberis*)

*S. uberis* has occasionally been reported associated with human infection. In most cases this was based on misidentification and *S. uberis* is not considered to be a human pathogen. Indeed, it is used as an ingredient in mouth wash. Likewise, *S. dysgalactiae* subsp. *dysgalactiae* does not affect humans (in contrast to *S. dysgalactiae* subsp. *equisimilis*).

Many environmental mastitis pathogens, e.g. *E. coli*, *Klebsiella*, *S. aureus* and *S. agalactiae* may also affect humans but there is no data on disease incidence in humans attributable to environmental mastitis. Likewise, there is no conclusive evidence to date that use of antimicrobials in dairy cattle has caused antimicrobial resistance (AMR) in mastitis pathogens with subsequent infections of humans.

In some countries, on-farm culture-based detection of mastitis pathogens is promoted. In other countries, there is concern about on-farm propagation of potential biohazards, and on-farm culture is discouraged.

GAP:

Culture-free on-farm diagnostics (see also Section 15)

## Risk of occurrence in humans, populations at risk, specific risk factors

Environmental mastitis (*E. coli*, *Str. uberis*)

Consumers of raw milk are at risk of ingesting milkborne pathogens, including but not limited to *Listeria monocytogenes*, non-typhoidal *Salmonella* species, *Campylobacter* species, *Yersinia* spp. and shiga-toxin producing *E. coli*. The presence of those pathogens in milk is generally the result of faecal or environmental contamination and not from shedding from mammary glands with mastitis. EU regulation prohibits the sale of visibly abnormal or adulterated milk, including the sale of milk containing antimicrobial residue, thus limiting the risk to food safety and public health.

Some studies suggest an association between *S. agalactiae* mastitis and colonization of the human throat, whereby both human-to-animal and animal-to-human transmission have been suggested, the latter through consumption of raw milk. Colonization of the human throat with *S. agalactiae* does not result in pharyngitis.

Toxin production by *S. aureus* (mastitis pathogen) or *Bacillus cereus* (environmental contaminant) may result in food poisoning, even if milk is heat treated or fermented, when toxins are heat stable.

GAPS:

Knowledge of patterns of raw milk consumption.

Diagnostic tests for detection of milkborne pathogens that pose food safety risks. Such diagnostics would need to cover different pathogen species than mastitis diagnostics as most mastitis causing species are not foodborne pathogens.

## Symptoms described in humans

Environmental mastitis (E. coli, Str. uberis)

Food poisoning (e.g. diarrhoea or vomiting), primarily due to foodborne pathogens of enteric origin as opposed to mastitis pathogens.

Listeria monocytogenes may cause abortion or stillbirth in pregnant women.

## Estimated level of under-reporting in humans

Environmental mastitis (E. coli, Str. uberis)

Most cases reported in the EU but possibly under-reported in developing countries.

## Likelihood of spread in humans

Environmental mastitis (E. coli, Str. uberis)

Highly unlikely when hygiene and quality standards are adhered to and milk is pasteurised prior to consumption.

## Impact on animal welfare and biodiversity

### Both disease and prevention/control measures related

Environmental mastitis (E. coli, Str. uberis)

Mastitis, particularly clinical mastitis, is painful and has a negative impact on cattle welfare. Reduced use of antimicrobials for prevention of mastitis in the dry period or for treatment of clinical mastitis may, at least theoretically, compromise animal welfare by increasing the risk of clinical mastitis in the dry period (demonstrated) or by reducing the probability or speed of cure (not demonstrated)

GAPS:

Impact of reduced use of preventive antimicrobials at dry off on incidence and cure of clinical mastitis and associated animal welfare.

Impact of selective treatment of non-severe clinical mastitis in lactation on animal welfare.

Impact of treatment of severe clinical mastitis without antibiotics.

### Endangered wild species affected or not (estimation for Europe / worldwide)

Environmental mastitis (E. coli, Str. uberis)

No.

### Slaughter necessity according to EU rules or other regions

Environmental mastitis (E. coli, Str. uberis)

Culling of affected cows may be needed for animal welfare reasons in cases of severe clinical mastitis, or to prevent transmission of infection in cases of chronic mastitis.

GAP:

Longevity of animals in the milking herd after environmental mastitis, as delineated by pathogen type, severity of infection and use of treatment products.

## Geographical distribution and spread

### Current occurrence/distribution

Environmental mastitis (E. coli, Str. uberis)

Ubiquitous worldwide with country-specific prevalence and incidence of pathogen genera and species as cause of subclinical or clinical mastitis. For example, *S. aureus* and *S. dysgalactiae* are relatively common as cause of mastitis in early lactation in some Nordic countries, whereas *E. coli* and *S. uberis* are more common as cause of mastitis in early lactation in the UK.

Annual nationwide surveillance of all farms exists only for *S. agalactiae* in bulk tank milk in Denmark.

GAP:

Systematic surveillance of prevalence and incidence of mastitis and its causes at national level.

### Epizootic/endemic- if epidemic frequency of outbreaks

Environmental mastitis (E. coli, Str. uberis)

Endemic.

## Seasonality

Environmental mastitis (E. coli, Str. uberis)

Seasonality differs between countries/climates and management systems, e.g. seasonal calving vs. year-round calving. Some pathogens are more common as cause of mastitis in housed cattle whereas other pathogens are more common in cattle on pasture, e.g. *S. dysgalactiae* and *S. uberis*, respectively, in The Netherlands but patterns differ between countries and systems. Summer mastitis, as the name suggests, is seasonally and driven by biting flies. Heat stress and pasture-based rations may contribute to increased incidence of mastitis in summer (Europe) whereas poor indoor climates may contribute to increased incidence in winter.

GAPS :

Interventions to prevent seasonal increase in incidence of pathogen-specific environmental mastitis.

Potential impact of climate change on environmental mastitis incidence including new demands on barn environment (ventilation).

## Speed of spatial spread during an outbreak

Environmental mastitis (E. coli, Str. uberis)

Because environmental mastitis is caused by organisms in the farm environment, notably those from bovine faeces, their spread is normally limited to the environment of an individual farm/herd or even a pen or management group within the herd. If an infected cow leaks milk, she may contaminate the environment and contribute to infections in other cows. Otherwise, cow-to-cow transmission would be considered contagious mastitis, not environmental mastitis. Within farms, outbreaks may occur over several days to months, depending on farm size, farm management and interventions. For example, introduction of wood- or peat-based bedding material that is contaminated with *Klebsiella* has occasionally resulted in mastitis outbreaks. Outbreaks affecting multiple herds are extremely rare and generally associated with the use of contaminated animal health products that have been distributed to multiple farms.

## Transboundary potential of the disease

Environmental mastitis (E. coli, Str. uberis)

Generally low, with the exception of Mycoplasma spp., which are considered to cause contagious rather than environmental mastitis and not described here.

## Seasonal cycle linked to climate

Environmental mastitis (E. coli, Str. uberis)

Yes; see Section “Seasonal cycle”

GAP :

Potential impact of climate change on environmental mastitis incidence

## Distribution of disease or vector linked to climate

Environmental mastitis (E. coli, Str. uberis)

Yes; see Sections “Vectors cyclical/non-cyclical”, “Current occurrence/distribution” and “Seasonal cycle”.

GAP :

Potential impact of climate change on environmental mastitis incidence

## Outbreaks linked to extreme weather

Environmental mastitis (E. coli, Str. uberis)

Heat stress or high humidity may contribute to immunosuppression and reduced host resistance to environmental mastitis, as well as increased environmental pathogen loads. We are not aware of evidence for heat waves, flooding or other extreme weather events causing environmental mastitis outbreaks in Europe.

## Sensitivity of disease or vectors to the effects of global climate change (climate/environment/land use)

Environmental mastitis (E. coli, Str. uberis)

Heat stress and humidity can increase the risk of mastitis by reducing host immunity and increasing pathogen load. Changes in herd management, e.g. prolonged housing seasons, may reduce the risk of accumulating pathogen loads in housed environments but they may alternatively increase the risk of other types of mastitis, as observed e.g. for S. uberis on pasture in the Netherlands.

GAPS:

Mechanisms contributing to increased incidence of mastitis during housing or on pasture, e.g. nutritional or climatological impacts on host immunity or pathogen exposure (faecal consistency and pathogen load). This is particularly true for new bedding types, such as recycled manure or biosolids.

Knowledge of how best to manage recycled waste as bedding particularly during hot and humid weather.

## Route of Transmission

Usual mode of transmission (introduction, means of spread)



Environmental mastitis (E. coli, Str. uberis)

Environmental mastitis is a disease entity defined by the environmental origin of the causative agents of mastitis.

## Occasional mode of transmission

Environmental mastitis (E. coli, Str. uberis)

Many species that may cause environmental mastitis may also cause contagious mastitis or, rarely, a hybrid form whereby the infected index animal causes contamination of the environment that subsequent results in infection of additional animals in the herd. In this case, the infected mammary gland causes additional infections not via the milking process (as is the case for contagious mastitis) but via the environment.

GAPS:

Markers to differentiate environmental pathogens that are unlikely to spread to other animals in the populations from members of the same bacteria species that do have the propensity to spread to other animals in the herd.

Farm and laboratory protocols for investigation of rare outbreaks, e.g. due to MRSA, S. canis, Pseudomonas, Serratia, or Klebsiella (rare in Europe compared to the USA).

## Conditions that favour spread

Environmental mastitis (E. coli, Str. uberis)

Imbalance between pathogen load and host resistance.

Pathogen load is primarily driven by bedding material and environmental hygiene, including beds/stall as well as alleys, walkways and parlour floors. The role of alleys and walkways, both indoors and outdoors, is generally underestimated, with primary attention given only to bedding. Poor ventilation and high humidity favour high pathogen loads and increased risk of mastitis.

Host resistance depend on genetics, teat morphology, nutrition (energy balance, minerals and vitamins), health status (e.g. immunosuppressive diseases such as bovine viral diarrhoea or BVD) and vaccination. In rare cases, teat injuries, e.g. due to poor hoof health, may contribute to high incidence of

*S. dysgalactiaemastitis*.

GAPS:

Best management practices for bedding material, notably recycled waste bedding whereby waste may be on-farm waste (recycled manure) or off-farm waste (e.g. recycled paper, recycled corn husks).

Availability and affordability of animal and user-friendly bedding material or systematic screening of potential alternative and sustainable bedding materials.

Detailed knowledge of the contribution nutrition, breeding or BVD eradication to control of environmental mastitis.

## Detection and Immune response to infection

### Mechanism of host response

Environmental mastitis (*E. coli*, *Str. uberis*)

Infection (invasion by bacteria followed by multiplication) is followed by inflammation, i.e. the host response. The host response is characterized by the classical signs of inflammation, which may manifest to varying degrees, i.e. rubor (redness), dolor (pain), calor (heat), tumor (swelling) and functio laesa (abnormal functioning, e.g. as manifested in serous, watery or flaky milk).

Endotoxin release from gram-negative pathogens may result in a very severe clinical response and associated sepsis due to disruption of the integrity of the blood-body barriers. Sepsis may be caused by organisms from the gut or lung rather than those from the mammary gland.

The inflammatory response is characterised by a massive influx of polymorphonuclear leukocytes. This response often fails to resolve infection, particularly for *Klebsiella*, *Strep. uberis* or *Staph. aureus*. Different pathogens have different ways of evading the host immune response, e.g. *S. uberis* appears to obstruct its uptake by PMNs by inactivating them, whereas *S. aureus* prevents uptake by binding the wrong end of antibodies whilst it also survives intracellularly after uptake by PMNs.

The host response may be delayed during negative energy balance or ketosis, as demonstrated *in vivo*, with a range of underpinning mechanism such as speed of chemotaxis and phagocytic capacity described *in vitro*.

## GAPS:

The role of the innate immune system in host response to mastitis is poorly understood, including but not limited to the role of macrophages, lymphocytes and cytokines. This is a gap in our knowledge that must be addressed to develop improved vaccines.

The feasibility of manipulating and strengthening the host immune response to mastitis through vaccination is poorly understood.

The role of adherence to mammary epithelial cells and invasion into mammary epithelial cells in vivo is unknown but this is primarily relevant to chronic and contagious mastitis rather than to environmental mastitis.

The most appropriate treatment modalities to support and strengthen the host response (antimicrobial, anti-inflammatory or supportive therapy) is poorly understood. Some treatments that were considered “essential” by practicing veterinarians for many years, e.g. udder lavage with colistin or use of 3<sup>rd</sup> or 4<sup>th</sup> generation cephalosporins, have been abandoned by others with no obvious negative impact. The necessity of antimicrobial treatment of environmental mastitis must be investigated in light of the societal pressure to reduce the use of antimicrobials, and mindful of the potential negative welfare impact of non-treatment.

## Immunological basis of diagnosis

### Environmental mastitis (E. coli, Str. uberis)

Diagnosis is primarily based on detection of inflammatory responses (e.g. colour, temperature and electrical conductivity of milk as monitored by milking machines; increase in milk somatic cell count or changes in differential cell count; California Mastitis Test; QScout; Foss) or detection of the pathogen, either through culture or through demonstration of the presence of pathogen DNA by Polymerase Chain Reaction (PCR; commercially available) or Loop-mediated Amplification (LAMP; experimental). Biomarkers of inflammation such as haptoglobin, NGase or LDH are also under investigation. In the 1980s, immunological diagnostics, e.g. based on ELISA were investigated but those have never been implemented due to lack of sensitivity and specificity.

## Main means of prevention, detection and control

## Sanitary measures

### Environmental mastitis (E. coli, Str. uberis)

Environmental hygiene, i.e. reduction of the pathogen load, is essential in prevention of environmental mastitis. See also Section “Route of transmission > Conditions that favour spread”.

Pre-milking teat disinfection is used in some countries and herds, depending on local regulation (e.g. acceptable levels of iodine in bulk tank milk) and management practices. Prior to teat disinfection, teats must be cleaned as disinfection is demonstrably ineffective when teats are contaminated with organic matter such as manure or bedding. Post milking teat disinfection reduce the risk of infection with numerous pathogens, whereby some teat disinfectants are primarily aimed at prevention of contagious mastitis by contact disinfection immediately after milking whereas others, e.g. so-called barriers dips, are primarily aimed at prevention of environmental mastitis and intended to provide protection not just immediately after milking but for a prolonged period between milkings.

### GAP:

Improved methods for pre- and post-milking teat cleaning and disinfection, particularly in automated milking systems.

## Mechanical and biological control

### Environmental mastitis (E. coli, Str. uberis)

Biological control, as in use of predatory insects or birds that control a pathogen or pest of interest, does not exist for environmental mastitis. Use of bedding conditioners to reduce bacteria counts may be a form of chemical (acidic or alkaline conditioners) or biological (e.g. bacterial control).

Breeding and feeding for increasingly high milk production per cow is often said to contribute to poor fertility and increased risk of production diseases such as environmental mastitis, thus impacting negatively on animal welfare, but evidence to support this claim is mixed.

Poor teat end condition or teat end callosity may increase the risk of mastitis. Teat end callosity can be prevented through appropriate adjustment and use of milking machines.

Mechanical control is essential for prevention of environmental mastitis, e.g. regular maintenance of bedding material to reduce pathogen loads and regular scraping of indoor alleys and walkways to reduce exposure to faecal organisms. Mechanical ventilation may contribute to reduced humidity and pathogen loads. Detailed guidance for management of bedding material is dependent on the type of material and the farm and climatological conditions.

A specific form of mechanical control consists of internal teat sealants, inert bismuth subnitrate paste inserted into the distal end of the teat at dry-off to prevent invasion of the mammary gland by environmental organisms. This form of mechanical control does not provide any curative effect, but it provides protection throughout the dry period, in contrast to external teat sealants, which tend to peel off.

#### GAPS:

Lack of knowledge of the feasibility of using phages or beneficial microbes as a means of biological control for mastitis pathogens in faeces or bedding.

Lack of guidelines for bedding management to reduce the risk of build-up of environmental mastitis causing pathogens, particularly for new bedding materials (e.g. manure, paper- or peat based).

Need for automated monitoring of teat end condition.

Role of mammary gland or teat end microbiota in reduction of the risk of environmental mastitis, and feasibility of manipulation of microbiota to reduce the risk of mastitis.

Role of production (milk yield) in susceptibility to environmental mastitis, and economic and biological feasibility of breeding and feeding for high production whilst reducing the risk of environmental mastitis.

## Diagnostic tools

Environmental mastitis (*E. coli*, *Str. uberis*)

Diagnostic tools can be divided into tool for detection of clinical mastitis, tools for detection of subclinical mastitis, and tools for detection of causative agents.

Diagnostic tools for detection of clinical mastitis consist of the senses (touch, sight; taste not recommended) and sensors with similar functions (temperature, colour, electrical conductivity). The main challenge for the use of senses is the time and skill needed, resulting in limited sensitivity (observations not made) whereas the main challenge for the use of sensors is the risk of false positives, resulting in limited specificity and false alarms. Even with a specificity of 99.9%, an alarm at quarter level would, on average, give a false alarm at every milking of a 250-cow herd. Additional markers in use or under development are derived from the host inflammatory response (see 8.2) and include LDH (lactate dehydrogenase), Hp (haptoglobin) and NGase (

lysosomal N-acetyl- $\beta$ -d-glucosaminidase).

Diagnostic tools for detection of subclinical mastitis are largely based on detection of elevated somatic cell count (SCC), with a variety of manual and automated on-farm and off-farm options available, including California Mastitis Test (manual, on-farm), QScout or DeLaval cell counter (automated, on-farm) and Fossomatic (automated, off-farm). Biomarkers such as NGAse may also be useful as indicators of mastitis.

At pathogen level, culture and DNA-detection are available. Culture can be conducted on-farm or off-farm. On-farm culture can be conducted using Petri dishes or Petri film. Increasingly, automated on-farm systems are being produced, e.g., both of which are based on detection of bacterial growth. Off-farm culture is offered by commercial diagnostic laboratories. DNA-based detection is commercially available in the form of PCR, which is used widely in some countries, e.g. the Nordic countries of Europe, whereas uptake is limited in many English-speaking countries, both in Europe and beyond. Elements contributing to such differences may include price, availability, animal health systems and predominant mastitis pathogens. LAMP-based assays for pathogen detection have been published but not yet commercialized. Biomarker-based assays for detection of pathogen-specific patterns of inflammation are also being studied.

#### GAPS:

Major gaps in diagnostics are both technical and social. There is an urgent need to understand the motivation of farmers and veterinarians for (non)use of diagnostic tools. Such research will inform on the feasibility and technical requirements of on-farm diagnostic testing. For example, research in the Netherlands has shown that diagnostic test results would need to be available within 8 hours (prior to the next milking in a 3X herd), rather than within 24 hours as previously assumed by some mastitis experts. It is unknown whether uptake of on-farm diagnostic could be improved if test results were available within 8 minutes, i.e. within the duration of a milking.

In addition, we need to understand the desirable level of discrimination, e.g. species level, genus level, or just gram-positive vs. gram-negative/no growth.

On the biological and technical side, there are gaps in sensitivity, specificity (false positive results due to contaminated samples), discriminatory power (number of pathogens or groups of pathogens recognized), ease of use, speed of detection, cost, integration in work flow, and integration with other farm data, e.g. about the individual quarter or cow.

## Vaccines

Environmental mastitis (E. coli, Str. uberis)

Vaccine availability differs between continents and may include commercially available and autogenous vaccines.

In Europe, commercial vaccines are available to protect against mastitis caused by *S. aureus*, *S. uberis* and *E. coli* in dairy cattle. There is strong evidence for the beneficial effect of the *E. coli* vaccine in terms of milk yield, cow welfare, and economic benefit, much less so for *S. aureus* whilst the *S. uberis* vaccine was only launched in 2018 so it is too early to pass scientific judgement on field efficacy.

#### GAPS:

Although vaccines are now available for the three major mastitis pathogens in Europe, further improvements are desirable, notably in the number of injections needed to obtain protective immunity, the level of protective immunity, and the method of administration (needle-free techniques would be desirable).

For other markets, additional vaccines would be desirable, e.g. a vaccine against *Strep. agalactiae* for Asia and South America.

## Therapeutics

### Environmental mastitis (*E. coli*, *Str. uberis*)

Intramammary infection caused by bacterial pathogens is routinely treated in lactation with short-acting intramammary antimicrobial infusion and, less commonly, with systemic administration of antimicrobials, e.g. in the case of severe clinical mastitis that may be accompanied by sepsis, or for lipophilic variants of penicillin G to achieve better distribution of active compound throughout the mammary gland tissue.

Inflammation is commonly treated with non-steroidal anti-inflammatory drugs (NSAID), which may reduce pain and improve appetite.

Supportive treatment may include administration of minerals (e.g. calcium, magnesium) and fluids, either orally or intravenously.

For prevention of intramammary infection during the dry period, long-acting intramammary antimicrobials are used routinely on many farms in many countries, historically with the exception of the Nordic countries in Europe. With growing societal concern about antimicrobial resistance (AMR) there is pressure to reduce the use of antimicrobials in animal agriculture, particularly the preventative use. This is leading to reduced use of blanket dry cow treatment (DCT), e.g. in the Netherlands, with limited negative impact on udder health. Of note, some antimicrobial products for DCT are registered for treatment of existing infections rather than for prevention of new infections, implying that blanket DCT constitutes off label use. For prevention of infections during the dry period, internal teat sealants are available (see Section "Main means of prevention, detection and control >Mechanical and biological control")

The World Health Organisation has classed some compounds as being Highest Priority Critically Important Antimicrobials, including fluoroquinolones and 3<sup>rd</sup>/4<sup>th</sup> generation cephalosporins (3/4CG). In some countries or veterinary practices, those compounds are used for treatment of clinical mastitis whereas they have been phased out elsewhere. There is some evidence that 3/4 CG enhance bacteriological and clinical cure of mild to moderate gram-negative mastitis,

whereas most other antimicrobial compounds for mastitis treatment do not. There is active debate about the need to treat mild to moderate gram-negative mastitis with antimicrobials, with a growing number of mastitis experts saying that such treatment is not beneficial to animal health and welfare and should be avoided to reduce the use of antimicrobials and any potential associated risk of selection for antimicrobial resistance. Likewise, treatment of culture-negative mastitis (no viable organism demonstrably present) or mastitis caused by *Mycoplasma*, yeast or algae (*Prototheca*) is considered unjustified. By contrast, antimicrobial treatment of gram-positive infections (e.g. caused by *Staphylococci* or *Streptococci*) enhances clinical and microbiological cure. Implementation of selective treatment of clinical mastitis would require the ability of on-farm diagnostics (see Section “Main means of prevention, detection and control >Diagnostic tools”)

Penicillin-resistant *S. aureus* is less likely to respond to treatment than penicillin-susceptible *S. aureus*, regardless of the antimicrobial compound used, and several laboratories offer routine testing for penicillin resistance. Antimicrobial treatment of PRS *S. aureus* is discouraged. Reports of PRS *S. agalactiae* are emerging from South America and China, but not from Europe. Macrolide resistance may occur in streptococci, but macrolides are not widely used for treatment, and their use in animals is discouraged by the WHO. Some rare mastitis pathogens (e.g. *Pseudomonas*, *Enterococci*) are commonly resistant to many antimicrobials and not responsive to treatment, whereas responsiveness or lack thereof for coliform organisms does not seem to be driven by AMR.

#### GAPS:

Alternatives to conventional antimicrobials, e.g. antimicrobial peptides such as Nisin or phages.

Potential role of microbiome modifiers as therapeutic agent.

Economic viability (cost-benefit analysis) of selective treatment of lactational mastitis.

## Biosecurity measures effective as a preventive measure

Environmental mastitis (*E. coli*, *Str. uberis*)

Prevention of contact between cows and other animal species, e.g. cats, dogs, pigs or wild birds, which may be reservoirs of mastitis pathogens.

There is anecdotal evidence that milkers may be a source of mastitis pathogens, e.g. *Staphylococcus epidermidis* or *S. agalactiae*. This risk can be reduced by wearing gloves during milking.



## Border/trade/movement control sufficient for control

Environmental mastitis (E. coli, Str. uberis)

Environmental mastitis is not seen as an impediment to movement. Historically, some countries (notably Denmark) restricted the movement of cattle from S. agalactiae-positive herds due to fears of contagious transmission. This policy has been abolished because it did not impact on incidence of S. agalactiae at herd level, contributing to the notion that environmental (human) sources of the pathogen are more important sources than infected cattle.

## Prevention tools

Environmental mastitis (E. coli, Str. uberis)

Prevention tools include sanitary measures, mechanical control, vaccines, biosecurity, breeding for resistance to mastitis, and nutrition (prevention of negative energy balance; adequate levels of vitamins, notably vitamin E, and minerals, notably selenium, copper and zinc).

## Surveillance

Environmental mastitis (E. coli, Str. uberis)

Within-herd surveillance or herd-health programmes are available and can be based on monitoring of somatic cell count and clinical mastitis with or without pathogen detection.

Veterinary practices and diagnostic laboratories that offer mastitis diagnostics can generate surveillance reports based on aggregated results at farm or laboratory level. Surveillance is passive and driven by sample submissions rather than active and unbiased.

In addition to pathogen surveillance, some laboratories and pharmaceutical companies conduct surveillance of antimicrobial susceptibility profiles.

Some laboratories offer routine testing of pathogen loads in bedding material.

#### GAPS:

Active, systematic, longitudinal surveillance of pathogens and antimicrobial resistance profiles across farming systems and countries.

Surveillance of environmental pathogen loads in bedding.

## Past experiences on success (and failures) of prevention, control, eradication in regions outside Europe

### Environmental mastitis (E. coli, Str. uberis)

Vaccines are used more widely in North America than in Europe, which is driven by a combination of economic and cultural factors. Udder health, which depends, among other things, on control of environmental mastitis is probably better in Europe than anywhere else in the world.

Despite a plethora of scientific knowledge regarding control of environmental mastitis and availability of a multitude of control tools and strategies, there is limited uptake of existing knowledge. At national level, udder health improvements or management changes appear to be driven primarily by regulatory requirements such as reductions in maximum bulk milk somatic cell count or antimicrobial use quota, respectively.

#### GAP:

Knowledge of socio-economic, cultural and behavioural drivers of uptake of control measures.

## Costs of above measures

### Environmental mastitis (E. coli, Str. uberis)

For decades, mastitis control has largely relied on use of antimicrobials. Prevention of mastitis (see 9.1, 9.2) requires use of alternative tools that rely on human labour or automation. Human labour is increasingly a limiting factor in bovine herd management and environmental mastitis control. In many countries,

animal husbandry and milking are jobs filled by immigrants because the local population in developed countries is not interested in working long hours of heavy labour in agriculture. In the most developed countries, human labour is replaced by automation (e.g. milking robots), driven by the economics of labour cost and availability vs. cost of equipment. This is a problem, for example, in Europe, North America and New Zealand.

#### GAPS:

Economic viability of mastitis prevention and control measures.

Automation to replace human labour in cow care and prevention and control of environmental mastitis, e.g. improved teat preparation, improved mastitis detection, automated monitoring and modulation of barn and bedding hygiene, automated detection of causative agents, and automated administration of vaccines or treatments.

## Disease information from the OIE

### Disease notifiable to the OIE

Environmental mastitis (E. coli, Str. uberis)

Not currently an OIE notifiable disease.

### OIE disease card available

Environmental mastitis (E. coli, Str. uberis)

No.

## OIE Terrestrial Animal Health Code

Environmental mastitis (E. coli, Str. uberis)

No.

## OIE Terrestrial Manual

Environmental mastitis (E. coli, Str. uberis)

No.

## Socio-economic impact

Zoonosis: impact on affected individuals and/or aggregated DALY figures

Environmental mastitis (E. coli, Str. uberis)

Not applicable.

Zoonosis: cost of treatment and control of the disease in humans

Environmental mastitis (E. coli, Str. uberis)

Not applicable.

## Direct impact (a) on production

Environmental mastitis (E. coli, Str. uberis)

Losses due to mortality vary by herd, pathogen and lactation stage, and range from very low to very high, with anecdotal reports of loss of 10% of the lactating herd in 3 months' time due to Klebsiellamastitis.

In addition to acute mortality, i.e. death due to clinical mastitis, there may be mortality due to culling of animals that do not recover from mastitis (continued impact on milk yield or quality) or that have poor reproductive performance due to mastitis.

Morbidity, measured as incidence, also varies widely, and ranges from less than 10 to more than 100 cases per 100 cow years.

GAP:

Cost-benefit scenarios for decision making (treatment and/or culling), relative to future production based on presentation of clinical state.

## Direct impact (b) cost of private and public control measures

Environmental mastitis (E. coli, Str. uberis)

Mastitis is among the most common and costly diseases of dairy cattle. In developed dairy industries, contagious mastitis is largely controlled and losses due to mastitis are increasingly caused by environmental mastitis.

In Europe, the average total costs of mastitis have been estimated at €240/lactating cow per year, in which failure costs (FC) contributed €120/lactating cow per year and prevention costs (PC) contributed another €120/lactating cow per year. Milk production losses, discarded milk, and culling were the main contributors to FC, at €32, €20, and €20/lactating cow per year, respectively. Labour costs were the main contributor to PC, next to consumables and investments, at €82, €34, and €4/lactating cow per year, respectively.

In the USA, the average case of clinical mastitis in early lactation (first 30 days) was estimated to cost of \$444, including \$128 in direct costs and \$316 in indirect costs. Direct costs included diagnostics (\$10), therapeutics (\$36), non-saleable milk (\$25), veterinary service (\$4), labour (\$21), and death loss (\$32). Indirect costs included future milk production loss (\$125), premature culling and replacement loss (\$182), and future reproductive loss (\$9).

Estimated costs vary widely with production systems and model assumptions.

Cost of mastitis control are largely borne by primary producers, and include morbidity and mortality as well as costs of treatment and prevention, e.g. labour, bedding material, mechanisation, animal health products, diagnostics, staff training.

GAP:

Knowledge of socio-economic drivers of mastitis control, both at value chain level and at farm level.

## Indirect impact

Environmental mastitis (*E. coli*, *Str. uberis*)

Farm gate milk price (price paid to farmers) and consumer price (milk price charged at retail, notably in supermarkets) are largely disconnected.

Milk price, product standards, expectations with regards to animal health and welfare, and pressure to reduce use of antimicrobials may place conflicting demands on dairy farms, e.g. high standards for animal health and welfare drive a growing need for control of environmental mastitis, pressure to reduce use of antimicrobials limits availability of current control tools for environmental mastitis, and milk price limits the economic viability of use of labour or automation as alternative control tools.

Intensification of production may mitigate some of the economic pressures on milk production and mastitis control through economies of scale, e.g. as seen in North American dairies with >10,000 head of cattle, but there is societal opposition to farms of that scale in Europe.

Small scale organic farming is promoted in civil society and European policy as an environmentally friendly alternative to conventional dairy farming, with reduced risk of antimicrobial resistance due to enhanced restrictions on antimicrobial use. Average yield per cow and per acre is lower for organic production than for conventional production, which impacts negatively on food security (availability and affordability of milk) as well as on the carbon footprint or climate

impact of milk production.

GAP:

Marco-economic predictions of the future viability of dairy farming in Europe.

## Trade implications

### Impact on international trade/exports from the EU

Environmental mastitis (E. coli, Str. uberis)

Milk quality is impacted by environmental mastitis, and milk quality criteria, e.g. with regards to somatic cell count, are important economic drivers of mastitis control. The pathogens contributing to environmental mastitis are ubiquitous world-wide and do not impact on trade or exports directly. Some EU countries, e.g. the Republic of Ireland and The Netherlands, have significant international export markets, e.g. for powdered milk. Environmental mastitis may impact on the ability to meet international quality and price expectations.

GAP:

None

### Impact on EU intra-community trade

Environmental mastitis (E. coli, Str. uberis)

Milk quality is impacted by environmental mastitis, and milk quality criteria, e.g. with regards to somatic cell count (SCC), are important economic drivers of mastitis control. In addition to SCC, criteria with regards to adulteration (presence of antimicrobial or medicinal residue, chemical contaminants such as iodine from teat dips) apply. The pathogens contributing to environmental mastitis are ubiquitous and do not impact on trade or exports directly. Milk buyers and processors may have quality criteria beyond those imposed by EU regulations. In addition, milk buyers and retailers may impose criteria with regards to animal welfare and antimicrobial use. High levels of environmental mastitis may impact negatively on animal welfare, milk quality and antimicrobial use.

GAP:

None

## Impact on national trade

Environmental mastitis (E. coli, Str. uberis)

See section “Impact on EU intra-community trade”.

GAP:

Role of consumers, retailers and processors in driving economic viability and choice of on-farm management of environmental mastitis, including treatment options and ensuing welfare implications.

## Main perceived obstacles for effective prevention and control

Environmental mastitis (E. coli, Str. uberis)



Despite a plethora of scientific knowledge regarding control of environmental mastitis and availability of a multitude of control tools and strategies, there is limited uptake of existing knowledge (see also Section “Main means of prevention, detection and control> Experiences / success (and failures) of prevention, control, eradication in other countries”

Labour shortage, including difficulties in staff recruitment, training, and retention (see also Section “Main means of prevention, detection and control>Costs of above measures)

#### GAPS:

Knowledge of socio-economic, cultural and behavioural drives of uptake of control measures.

Increased automation of dairy cattle management and milk harvesting.

## Main perceived facilitators for effective prevention and control

Environmental mastitis (E. coli, Str. uberis)

Milk price.

Animal health and welfare and quality standards as imposed by national or international regulatory bodies or milk buyers.

#### GAPS:

Availability of preventive tools and efficient therapeutic protocols.

Knowledge of socio-economic, cultural and behavioural drives of uptake of control measures.

## Risk

Environmental mastitis (E. coli, Str. uberis)

Antimicrobial use in animal agriculture is perceived to contribute to selective pressure which may favour survival of resistant pathogens and contribute to the public health crisis attributed to antimicrobial resistance.

As the world population grows and becomes wealthier on average, demand for animal protein, including from dairy products, is growing, particularly in Asia. Already, in some European cities, limits are placed on the number of tins of powdered milk that can be purchased per person to avoid tourists emptying the shelves, leaving locals without access to the product, even if it is produced within the country. At the same time, there is concern about climate change and availability of land and water. Hence, there is a need for “Sustainable Intensification”, producing more with less to ensure food security (routine availability and affordability of safe and nutritious food for everyone) whilst limiting environmental impact of dairy food production.

Environmental mastitis limits the production efficiency of dairy cattle, as well as their health and welfare. Thus, there are multiple societal incentives to prevent and control environmental mastitis. At the same time, however, there are several societal developments that put pressure on sustainable intensification, including but not limited to labour shortage, objections to large farms, concerns about animal welfare and biodiversity, perceived environmental benefits of organic farming, and a demand for reduced use of antimicrobials in animal agriculture. Whilst technical developments, including but not limited to automation, new vaccines, new diagnostics and new treatment products, may contribute to sustainable intensification and long-term viability of the dairy industry, it is crucial that socio-economic and environmental (dis)incentives and drivers are given due consideration too.

GAP:

There is no evidence for or against the assumed contribution to AMR as a public health or medical problem for antimicrobial use associated with the control of environmental mastitis.

Environmental and economic impact assessment of environmental mastitis as limiting factor in dairy food production at national, supranational and global scales

## Main critical gaps

Environmental mastitis (E. coli, Str. uberis)

Information on host-pathogens interactions are still very few and controversial. Studies on these aspects are needed both to identify the most relevant virulent strains and to identify methods which will enable the udder to reduce its susceptibility, independently from the bacteria involved.

# Conclusion

Environmental mastitis (E. coli, Str. uberis)

Whilst the use of dry cow and lactating cow intramammary antibiotics is acceptable to conventional dairy farmers it is more difficult for organic dairy farmers and their animals may well act as a reservoir for the organisms involved. The use of the teat sealant by organic farmers is acceptable as it is with conventional dairy farmers but there is a potential problem with teat sealing if there is already a sub-clinical infection in the quarters. Stress and reduced immunity for example due to a leucopenia caused by other infections can cause recrudescence particularly of sub-clinical cases.

# Sources of information

## Expert group composition

Environmental mastitis (E. coli, Str. uberis)

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Environmental mastitis (E. coli, Str. uberis)

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## Date of submission by expert group

Environmental mastitis (E. coli, Str. uberis)

30 January 2019

## References

Environmental mastitis (E. coli, Str. uberis)

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