

REPORT FROM THE
**4th GLOBAL MILK QUALITY
EXPERT FORUM**



Boehringer
Ingelheim





The **Global Milk Quality Expert Forum** is organised and supported by Boehringer Ingelheim, to encourage the exchange of forefront ideas and concepts between researchers and practitioners on a global level. The GMQEF allows the meeting of world experts on milk hygiene and udder health for in-depth discussions on current issues. Its aim is to meet on a yearly basis to share knowledge and expert opinion.

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Chatham House rules report

THE ROLE OF THE MILK MICROBIOME IN MASTITIS IN DAIRY COWS

“*Mastitis: inflammation, dysbiosis or infection?*” was the topic of the 4th Global Milk Quality Expert Forum, held on 11 and 12 January 2018 in Milano, Italy, and organised by animal health company Boehringer Ingelheim.

Chaired by Giacomo Tolasi, an independent veterinary consultant from Italy, the programme included presentations by Georgios Oikonomou (University of Liverpool, UK), Harro Timmerman (Boehringer Ingelheim) and Satu Pyörälä (University of Helsinki, Finland). Workshops held on the second day of the meeting allowed for an excellent interaction between participants and speakers. The forum was attended by a total of 11 milk quality experts from different countries.

Microbiome: diversity is key

The presence of microorganisms in cow milk as measured by genetic profiling (16SrDNA sequencing), the so-called milk microbiome, was at the heart of discussions at the meeting. George Oikonomou (University of Liverpool), one of the pioneers working on the bovine milk microbiome, presented a number of studies highlighting its importance in udder health.

Contrary to popular belief, milk is not sterile, even in healthy milk glands. In human medicine, there now was a general consensus that healthy women’s breast milk contains a constituent microbiota, as demonstrated on biopsies. Microbiome richness and diversity are two important concepts worth taking a second to fully understand. According to studies, the composition of this flora changes during the lactation period and is influenced by factors such as the woman’s weight and mode of delivery. The human milk microbiome was found to be linked to the faecal microbiome of the offspring. However, it is unclear whether this is due to environmental contamination or due to a so-called enteromammary pathway.

Microbiome richness and diversity are two important concepts worth taking a second to fully understand.



The impact of antibiotics

Studies in dairy cows showed that healthy quarters not only had a lower bacterial load, but also a much more diverse microbiome than quarters with mastitis. *Streptococcus uberis* furthermore seemed to be a standard component of the healthy microbiome while *Escherichia coli* was only present in cows with coli-mastitis.

The composition of the microbiome also determined how the udder reacted to infection – and to treatment. In a comparative study, experimentally infected quarters were treated with an intramammary 3rd-generation cephalosporin (ceftiofur) and compared to untreated controls. Ceftiofur had an effect on the bacterial load in these models, but not on microbial diversity, somatic cell count or bacterial clearance. While the bacterial load in the milk decreased with this broad-spectrum antibiotic treatment, it took 14 days to clear out *E. coli* – regardless of whether the quarter was treated or not.

Many questions remain unanswered, however. In experimental infections, only very few bacteria (<100 CFU) are needed to induce mastitis. However, the udder does not respond with an immune reaction to the healthy microbiome. It is suggested that the immune system recognises these bacteria (and in these numbers) as innocuous commensals.

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“The composition of the microbiome determines the udder’s reaction to infection – and to treatment.”



Not challenged, not treated

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Not challenged, treated

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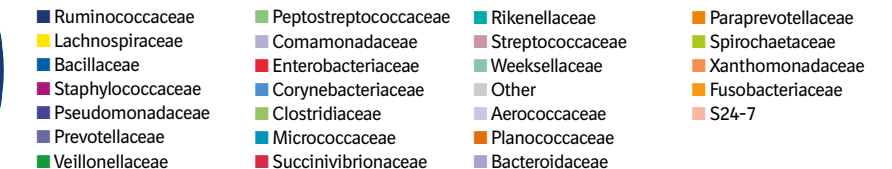
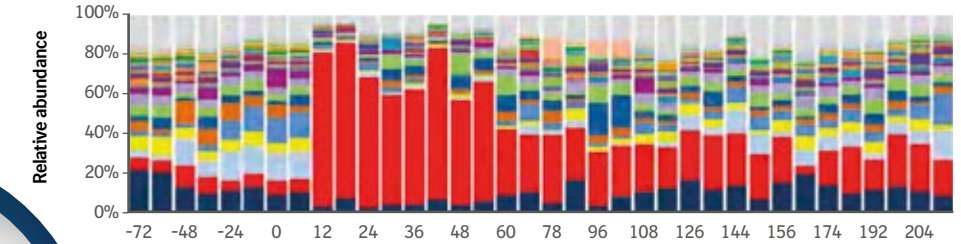
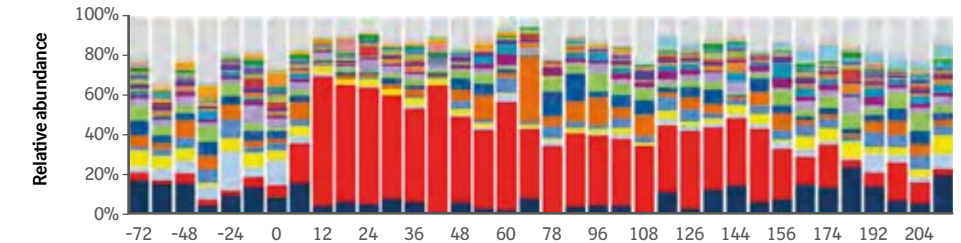
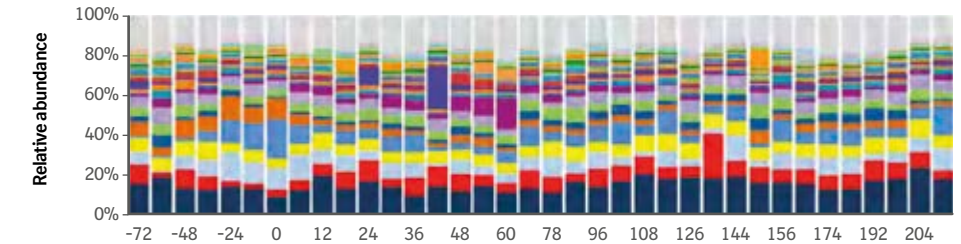
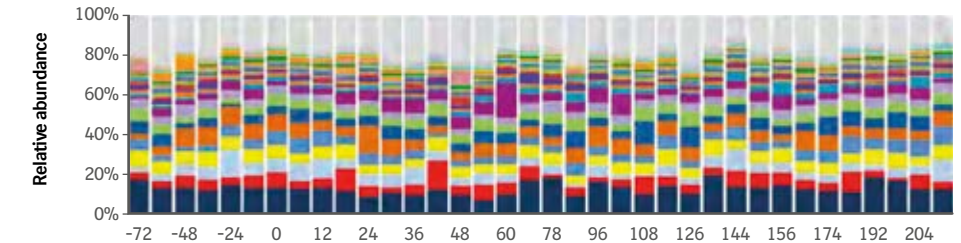
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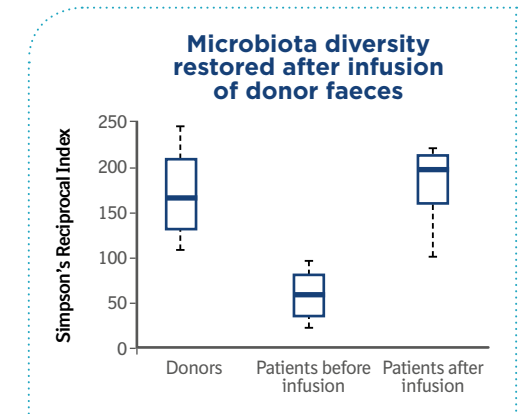
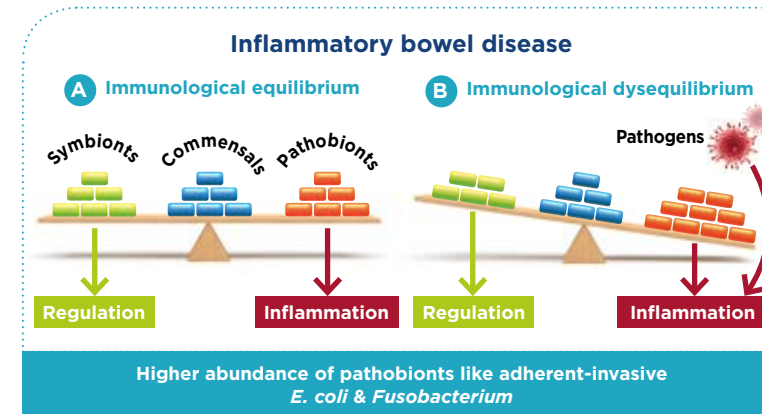
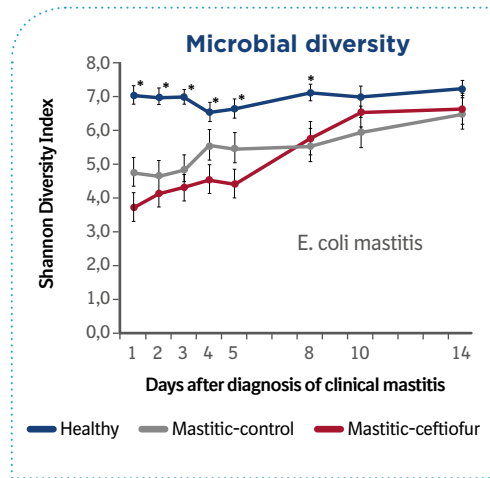
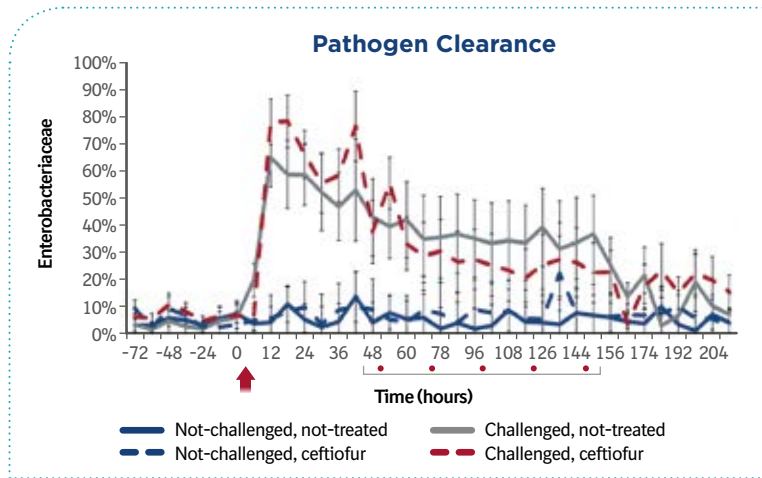
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Challenged, treated

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With a more high richness and diverse microbiome, a much more capable and resilient immune system may be present.





Ganda et al., 2017

Dysbiosis: a key feature of infection, inflammation and immune dysregulation

Studies in humans further highlighted the potentially protective role of the microbiome, live therapeutics and probiotics. Harro Timmerman, a specialist in microbiology and immunology at Boehringer Ingelheim presented the highlights of 18 years of microbiome research.

A study of infants in the first 12 weeks of life showed that the development of the gut microbiome followed a set pattern. The first colonising bacteria in the gut were those from the birth canal, followed by those of the skin (Enterobacteriaceae, staphylococci and streptococci). These bacteria consume oxygen and thereby pave way for the obligate anaerobes, such as Bifidobacteriaceae. The guts of these infants also showed a fluctuating abundance of pathogens, although this was rarely associated with disease. *Clostridium difficile* was only found in formula-fed infants, and it was suggested that breast milk has protective factors preventing its colonisation.

While on average 5.6 pathogens were found in diarrhoea samples of young infants, samples of healthy infants still contained more than 4 pathogens, a Bangladeshi study found. This suggested that diarrhoea was a multi-pathogen condition. A correlation exists between pathogen load and occurrence of disease: diarrhoea was associated with pathogen excess. Dysbiosis was found to be a key feature of infection, inflammation and immune dysregulation, characterised by a loss of diversity, pathobiont overgrowth and a loss of commensal obligate anaerobes.

Restoring the microbiome

Studies of human mastitis cases showed that microbiome diversity decreased with disease severity, although it was not clear if this was due to a loss of symbionts (the 'good guys') or a consequence of emerging pathobionts (the 'partners in crime', usually commensals 'having turned sour') – or both. In inflammatory bowel disease, this was well researched, with symbionts driving the regulatory immune cells and pathobionts the inflammatory immune cells.

It was suggested that therapeutic strategies should target the pathobionts while supporting the symbionts to help restore the healthy microbiome. Therapy based on the restoration of the commensal population was not new, participants learned. In fact, old-school farriers sometimes treated sick horses with 'poo tea' extracted from faecal material of healthy horses. Rumen transfaunation has also been used to treat animals that had ingested plant toxins. A Dutch study from 2013 showed that duodenal infusion of healthy donor faeces greatly improved the

Dysbiosis is a state in which there is an imbalance of microorganisms within our bodies. When in balance, these colonies of microorganisms tend to have a favorable effect. When there is an imbalance, we may experience unwanted symptoms.





cure rate for the treatment of recurrent *Clostridium difficile* – from 30% with vancomycin therapy to >90% after faeces infusion – while restoring gut microbiome diversity.

While live therapeutics such as probiotics and faecal transplants had clearly proven their worth in human medicine, the effect of oral probiotics is probably difficult to achieve in ruminating cattle.

“Studies of human mastitis cases showed that microbiome diversity decreased with disease severity”

Milk microbiome vs. udder microbiome

While bacterial culture is still the gold standard for the examination of cow milk, results are usually restricted to a maximum of two species, otherwise the sample is considered ‘contaminated’. Using PCR typically reveals the presence of multiple species, not just due to the more sensitive technique but also because the sample used was ten times larger than for culture. Satu Pyörälä, mastitis specialist and professor emerita of the University of Helsinki, highlighted a few critical points to be considered in microbiome research.

But results are not only influenced by the diagnostic technique used, but also by sampling. Samples obtained directly from the milk gland cistern using a closed cannula system through the skin (with vacuum tube, directly from the milk chamber) were found to contain no or only a single species of microorganism compared to conventional sampling through strip milking. Microbiotic diversity also differed between conventional sampling and needle aspiration: needle samples contained more proteobacteria while conventional samples contained a larger proportion of actinobacteria – mainly skin commensals. Studies have shown that streptococci were relatively more abundant in conventional sampling and staphylococci relatively more prevalent in samples obtained via percutaneous needle aspiration.

It was important to remember that ‘milk microbiome’ does not necessarily equal ‘milk gland microbiome’. Given the udder’s open system, with bacteria going in and out, milk microbiota could originate from the udder, the environment (faeces, forage, bedding), the skin and/or the teat end.

Group discussions

Workshops held on the second day of the meeting allowed for an excellent interaction between participants and speakers.

Below are a few excerpts and quotes:

Further research on milk microbiome is needed to understand better how influence the health status in the udder.

“ This is a paradigm shift. We thought normal milk did not contain bacteria; now we see that the whole udder is colonized with commensals that do not result in an inflammation. The fact that *Streptococcus uberis* is present in all milk samples is surprising. We used to tell our farmers they have to be more hygienic when we found *uberis*, now we see that it is part of a normal udder.

“ In my daily work as a practitioner this does apply to me but it will not change my daily practice - yet. Probably in a few years we can have more information on how we apply this.”

“ Maybe the presence of a microbiome contributes to explaining why we still cannot control mastitis; why we so often see disappointing treatment results? Take teat dipping for instance; maybe we are influencing the teat end microbiome too much? Maybe by understanding this we can be more successful? ”

“ Stressed cows make *E. coli* express different virulence factors. Maybe stress in a cow can make the bacteria turn sour? We are missing the other factors: heat stress, ketosis, etc. We know a bit about ketosis and how it changes immunology, but do not for acidosis. There are so many missing links in between... ”

“ Pathobionts are commensals that turn sour - maybe all bacteria are Dr Jekyll and Mr Hyde at the same time. ”

“ We thought we were successful using antibiotics, but it might be that success is based on changes to the microbiome - without us knowing. ”

CONCLUSION

With few exceptions, the experts at the meeting acknowledged the existence and role of bovine milk microbiome, but whether it originates from the teat canal, environment or from inside the udder remained a point of debate. Microbiome research has caused a paradigm shift in how scientists view health and disease in humans – and similar insights are likely to change the therapeutic approach to mastitis in the near future.

As one of the group members concluded:

“If it is true, it will change everything we have been doing – I’m very excited!”

Speakers

Georgios Oikonomou, University of Liverpool
Harro Timmerman, Boehringer Ingelheim
Satu Pyörälä, University of Helsinki

Participants

Giacomo Tolasi, Italy (Chair)
Martin Pol, Argentina
Ian Hodge, New Zealand
Sarne de Vlieghe, Belgium
Bill May, UK
Michael Farre, Denmark
Olivier Salat, France
Oriol Franquesa, Spain
Volker Krömker, Germany
Tine van Werven, The Netherlands
Marcos Veiga, Brazil
Elke Abbeles
 (Boehringer Ingelheim)
Thomas Manske
 (Boehringer Ingelheim)
Anne Charlotte Christensen
 (Boehringer Ingelheim)

Previous GMQF events

2015 (Mechelen, Belgium):
 Prudent use of antibiotics
2016 (Copenhagen, Denmark):
 Is dry cow therapy here to stay?
2017 (Barcelona, Spain):
 The benefit of extended therapy for mastitis,
 myth or fact?

