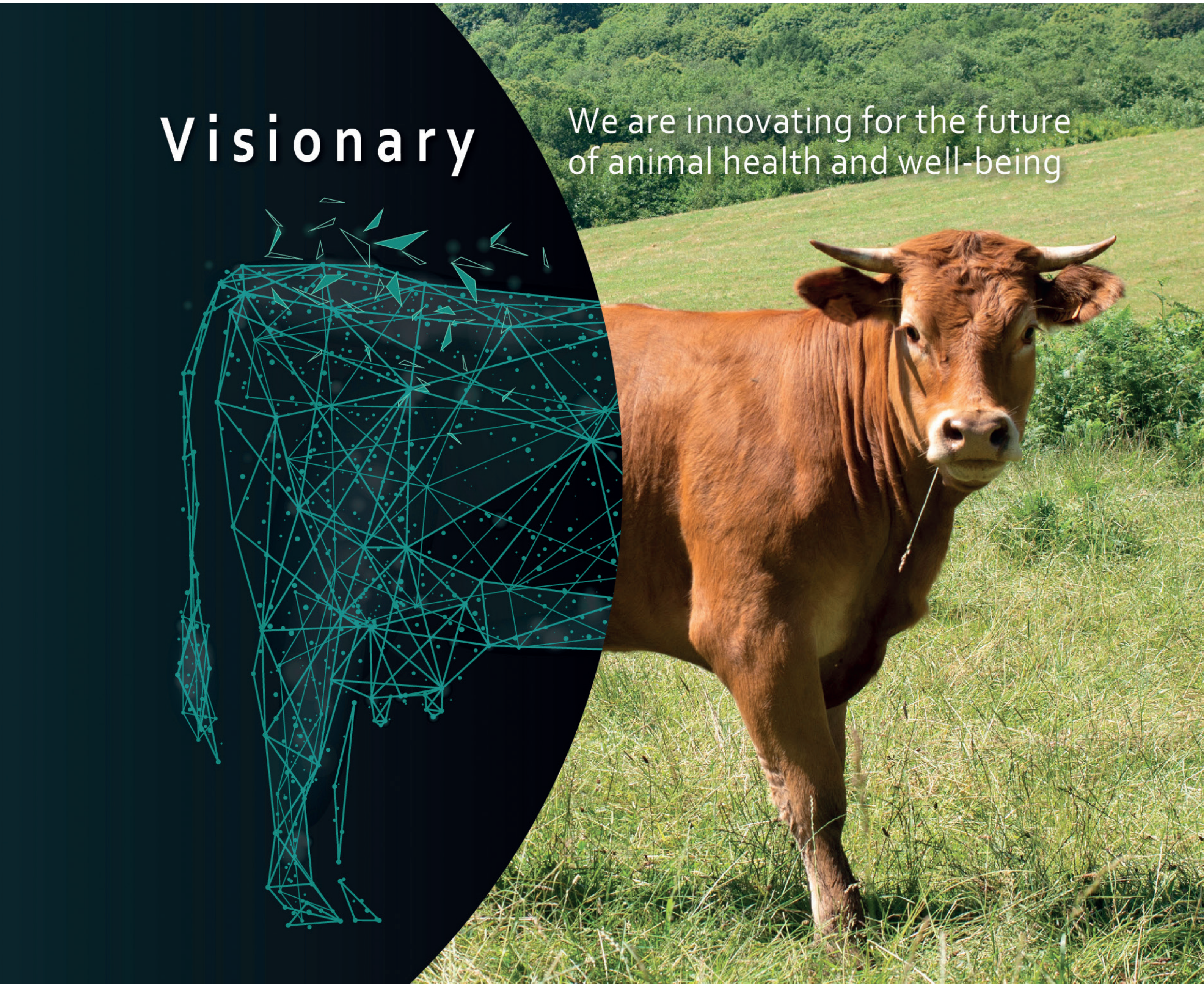
MSD ANIMAL HEALTH'S

TECHNICAL SYMPOSIUM 2022

31 October – 1 November 2022

SCIENCE TO BEST PRACTICE

National Library of New Zealand, Wellington



Welcome

Welcome to MSD Animal Health's Livestock Technical Symposium.

MSD prides itself on delivering a wide range of world-class innovative products that help veterinarians, pet owners and farmers care for their animals. Backed by heavy investment into research and development, not only do we invest in new products, but also our current product portfolio, in order to ensure all of our products remain at the top of the game and are effective and sustainable.

Since our last symposium, MSD Animal Health has undergone considerable change and growth by welcoming Allflex into our business. Aligned and guided by one mission 'The Science of Healthier Animals™' our expanded business now delivers market leading technology and intelligence to both the dairy industry and the sheep and beef sector.

All MSD and Allflex products are supported by our New Zealand team of regional veterinarians, who have a huge wealth of knowledge and understanding of the challenges of New Zealand livestock veterinarians and New Zealand farmers. Our local team is further supported by a strong global technical team, allowing New Zealand to tap into world-class science around animal health, wearable technology and milking intelligence.

Over the next two days you will hear about MSD's latest research and innovation regarding disease prevention and technology, both locally and globally. In addition you will have an opportunity to hear from key speakers from the wider New Zealand veterinary community, as well as local practitioners with interesting case studies.

We hope this will provide you, as practicing veterinarians, information and ideas that you can take back to your business to help with evidence-based decision making as well as creating new opportunities with your farming customers.

I look forward to an interesting couple of days and hope this symposium will be of value to you and your practice.

Kind regards,

Penny Mehrtens.
Livestock Technical Lead

DAY 1 – Monday 31st October		
Time	**Talk**	**Speaker**
10.00am – 10.30am	**MORNING TEA**	
10.30am – 10.45am	Introduction	Pauline Calvert
10.45am – 11.15am	Technology and Intelligence, Blue Skies	Guy Fridkovski (MSD Animal Health Intelligence)
11.15am – 11.45am	Chlamydia pecorum	John O'Connell (MPI)
11.45am – 12.30pm	Chief Veterinary Officer report	Mary van Andel (MPI)
12.30pm – 1.15pm	**LUNCH**	
1.15pm – 1.30pm	LIC Diagnostics Update	Mike Wilson (LIC)
1.30pm – 2.30pm	Sheep and Beef Updates	Anne Ridler (Massey University) / Kim Kelly
2.30pm – 3.00pm	Clostridial Case Report	Cosmin Susa (Hunterville Veterinary Club)
3.00pm – 3.15pm	**AFTERNOON TEA**	
3.15pm – 3.45pm	Yersinia in Cattle	Jackie Benschop (Massey University) / Kim Kelly
3.45pm – 4.15pm	Salmonella AMR Study	Chris Compton (Massey University) / Jo Holter
4.15pm – 5.15pm	MSD Research Update	Jo Holter / Jantijn Swinkels
6.30pm	**DINNER**	**Thistle Inn, 3 Mulgrave Street, Thorndon.** Walking bus leaving Atura Hotel at 6.15pm

DAY 2 – Tuesday 1st November		
8.30am – 8.45am	Intro - Monitoring	Steph Voice / Austin Heffernan
8.45am – 9.15am	ShutOut ITS Study plus global update	Rico Nortje (Rangiora Vet Centre) / Penny Mehrtens
9.15am – 10.00am	MPI Endemics Team BVD	Kat Govender / Colin O'Connor (MPI)
10.00am – 10.15am	**MORNING TEA**	
10.15am – 11.00am	Cell Sense SCC Study	Scott McDougall (AnexaFVC / Cognosco) / Rob Orchard (MSD Animal Health Intelligence)
11.00am – 11:30am	Mastitis Management using Milk sensors	Rob Orchard (MSD Animal Health Intelligence)
11.30am – 12.00pm	Monitoring Case studies	Rico Nortje (Rangiora Vet Centre)
12.00pm – 12.45pm	**LUNCH**	
12.45pm – 1.30pm	Learnings – springer/ transition cow management	Ryan Luckman (Oamaru Vets)
1.30pm – 1.45pm	Closing	Penny Mehrtens
2.00pm – 4.00pm	**EXTRA** – Cow collar custom report building workshop	Steph Voice, Amanda Kilby, Nicole Cooper, Alex Smith

Chlamydia pecorum abortion in hoggets

John O'Connell, MPI

Chlamydia (Chlamydophila) pecorum is an obligate intracellular bacterium with pathogenic and non-pathogenic strains and is found in a wide host range among domestic animals and wildlife. In Australia it causes conjunctivitis and urogenital disease in Koalas – a major threat to their very survival. It causes polyarthritis and keratoconjunctivitis in calves and lambs overseas and sporadic bovine encephalomyelitis (SBE) in calves worldwide (including New Zealand). It has been reported as a sporadic cause of abortion in sheep, goats, and cattle overseas. However, it has recently been implicated in ovine abortion storms in New Zealand and Australia.

At the 2019 New Zealand Veterinary Association (NZVA) conference Sarah Williams of North Canterbury Vet Clinics (Culverden) presented on the first *C.pecorum* associated abortion storm in a New Zealand flock – an outbreak that MPI assisted Sarah and her commercial veterinary laboratory in investigating. In 2019 MPI again assisted in confirming the involvement of *C.pecorum* in three South Island ovine abortion storm investigations.

In preparation for the 2020 season, MPI distributed an information sheet through the commercial veterinary laboratories and the NZVA, to increase awareness among veterinary practitioners of the association of *C.pecorum* with ovine abortion storms and to seek their assistance in determining if *C.pecorum* could be a more widespread cause of ovine abortions. MPI funded PCR and immunohistochemistry testing where a submission met certain criteria including a full range of appropriate samples being submitted in accordance with commercial laboratory guidelines. This resulted in two more *C.pecorum* associated abortion outbreaks being confirmed – this time in the North Island. There were no *C.pecorum* associated abortion outbreaks diagnosed in the 2021 season.

In total *C.pecorum* associated abortion had been confirmed in 5 flocks over three years – three in the South Island and two in the North Island. In two flocks it has occurred in two consecutive years. In four flocks it has affected hoggets. The other was a mixed age / two tooth flock. Abortions commenced five to seven weeks before the planned start of lambing. Retained placentas, sick ewes or ewe deaths were not a feature. Lambs could be any of well developed, fresh, decomposed, or mummified. Grossly, some but not all placentas showed mild inter-cotyledonary thickening with pale white opaqueness. On histopathology, lesions can be present in various tissues including the inter-cotyledonary placenta. While not pathognomonic, the placental histopathology lesions can be suggestive of *C.pecorum* involvement. There were two submissions that were *C.pecorum* negative on PCR of stomach contents. However, when suggestive placental histopathology was further investigated with MPI funded immunohistochemistry, *C.pecorum* was confirmed in the placental lesions. In all outbreaks the abortion cohort went on to have expected reproductive performance the following year.

Comparison of *C.pecorum* antibody titres between aborted hoggets and non-aborted hoggets from one flock, from 2020, yielded a serological profile that suggests there could be a place for serology in investigating *C.pecorum* associated abortion outbreaks.

In 2022 MPI made funding available for *C.pecorum* PCR at the commercial laboratories, should investigation for the usual aetiological agents (following submission of the full range of appropriate fresh and fixed samples) fail to arrive at a diagnosis, or if requested by a pathologist based on histological evidence. Of most interest to MPI, would have been the identification of a PCR positive flock, for serological investigation. However, at the time of writing (September 2022), no *C.pecorum* attributable abortions have been reported.

References

O'Connell J, Bestbier M. Chlamydia pecorum associated ovine abortion – an update for veterinary practitioners, *Grazing Gazette*, 59, 30-31, 2021.

Williams S. The challenges of an unusual abortion outbreak in a ewe flock. *Proceedings of the Society of Sheep and Beef Cattle Veterinarians of the New Zealand Veterinary Association*, 69-74, 2019.

Westermann T, Jenkins C, Onizawa E, Gestier S, McNally J, Kirkland P, Zhang J, Bogema D, Manning LK, Walker K, Pinczowski P. Chlamydia pecorum-associated sporadic ovine abortion (2020) *Veterinary Pathology*, 1-9, 2020.

Preparing for Foot & Mouth Disease in New Zealand

Mary van Andel, MPI

On the 9th of May 2022 Indonesia formally declared two outbreaks of FMD to the World Organisation for Animal Health (formally the OIE). The strain of the virus has been identified as serotype O of IND2001, a strain that is common in the Southeast Asian region. Indonesia had previously been free of FMD for more than 30 years.

A National State of Emergency was declared for FMD on June 29th 2022. Reportedly, this will stay in force until 31st December 2022. Recorded cases of the disease appear to be moving from west to east after initial detection in the west. Police and the Indonesian Military have been engaged in the vaccination of animals and enforcement of movement control as a result of the state of emergency.

The report of Foot and Mouth disease (FMD) in Indonesia in May this year has presented an opportunity to relook at our FMD preparedness, and wider border protection measures.

Whilst the risk of FMD introduction to New Zealand hasn't changed greatly with the Indonesian outbreak, it is a lot closer to Australia, and there is the opportunity to reassess our plans in light of the learnings and recommendations from both the Mycoplasma bovis eradication programme and New Zealand's all of government response to COVID-19.

A dedicated FMD taskforce for readiness is updating our existing plans to create a blueprint for a FMD response and further accelerate our level of preparedness. The taskforce has a dedicated director, governance mechanisms and leadership team and comprises subject matter experts from across MPI. The taskforce is working closely across animal sector industry bodies, Government Industry Agreement (GIA) partners for biosecurity readiness and response and other government agencies.

The work programme addresses four key areas:

1. building resilience (across MPI, government agencies and in the animal industries)
2. keeping the disease out of New Zealand
3. stamping it out should it arrive here
4. recovery from an outbreak.

FMD would affect all of us in New Zealand should it arrive here. The impact on farmers, rural communities, primary producers and the economy would touch all of us.

Helcococcus ovis – another (unwanted?) first for New Zealand

Kim Kelly, MSD Animal Health & Louise Hughes, VetSouth Gore.

Introduction

A Southland farmer who uses the same vaccines every year in his hoggets, called the clinic 6 days after vaccinating, upon discovering some hoggets with large lumps and many were lame. In total 52 out of 560 were affected over the subsequent 3 weeks. There were 8 deaths (including two sacrificial PM's). *Helcococcus ovis* was cultured – an unusual bacteria in New Zealand and the first case presenting like this in the world! Assumption was the bacteria was dragged into the muscle from skin via the vaccination needle, as thorough investigation into the batches of vaccine involved ruled out any direct involvement.

History

The hoggets were not up to date with clostridial vaccinations, they had received only 1 dose at weaning (December). They were shorn 6 days before vaccination. They were yarded for 3 hours maximum to be vaccinated and drenched. All the correct techniques were said to be used and the hoggets were clean and dry. The vaccines and drench were reported to have been stored correctly. A new vaccinator gun was used for one product, a used but clean one for the other. The drench gun was calibrated before beginning. The needles were appropriate length and changed about every 100 hoggets. This is less often than recommended, but it is a common rate of needle change.

The vet visited the farm and examined all the animals which had swellings or were lame. Several had swellings over their rump, others had swelling at the injection site, some had both. 7 were lame in a forelimb, 16 lame in a hindlimb and 11 had brisket swelling. The hoggets were in some cases extremely lame and had high temperatures (39.8 - 42.1 °C), pitting oedema, diffuse swelling, and the affected areas were hot to touch. One animal was euthanised at the first visit (recumbent) and fresh and fixed muscle and organ samples, blood samples, oedematous fluid and samples of 'pus' from swollen areas obtained from this animal, as well as some fluid from remaining lumps, were sent to Gribbles for testing. Affected animals were treated with antibiotics (Bivatop®) and anti-inflammatories (Metacam®).

At the second visit a week later further samples were taken, and also one severely affected hogget was euthanised on humane reasons. Lots of photos were taken– a reminder this is essential for all thorough case investigations. Some of the hind limb/rump swellings had burst out since first visit (see photos).

Lab results

The oedematous fluid from the first visit came back as *"Heavy growth of Helcococcus ovis after 48 hours (Organism identification includes use of MALDI-TOF MS at referral laboratory)"*. No anaerobes or any other bacteria were isolated. Sensitivities were checked and the bacteria was sensitive to most antibiotics but resistant to trimethoprim sulpha.

This unusual result led to a quick phone call to the friendly local pathologist who was likewise intrigued with this case. Histology was completed on areas around the fluid with a diagnosis of a severe subacute necro-suppurative, bacterial myositis. In some of these areas there were large bacterial colonies. Gram stain showed massive numbers of gram-positive cocci resembling *Helcococcus ovis*.

The second visit samples also revealed some fluid samples with *Helcococcus ovis*, but in addition there were other samples that cultured a heavy mixed growth with *Trueperella pyogenes* dominating. This would fit more with standard injection site infections. Interestingly there were no anaerobes isolated. The histology result at this time indicated the inflammatory reaction was at a walled off, recovery stage but still active with now mixed population of secondary invaders. The vascular changes in the muscle layer at that stage were most likely secondary.

Discussion and Conclusion

The bacteria *Helcococcus ovis* is a gram-positive coccus, facultatively anaerobic and catalase negative. It was first described in sheep in Spain with mastitis in the late 1990's in combination with *Trueperella pyogenes*[1]. Since then, it has been co-isolated from cattle suffering from different diseases such as valvular endocarditis[2], metritis[3] and associated with bovine abortion cases[4]. In human medicine, *Helcococcus ovis* was isolated from an artificial eye infection[5]. In all these reports, co-infections with *T. pyogenes, Streptococcus spp., Staphylococcus aureus* or *E. coli* were present. In 2020 in a case of suppurative arthritis in a four-week-old lamb in Australia, *Helcococcus ovis* was grown in pure growth, as in this case[6]. Massey University has cultured this as an incidental bacteria twice in ewe's udders, but this is the first time in NZ it has been linked to pathology or found outside of a research context.

It is now described as 'an emerging pathogen' and should be considered whenever unusual cases present. It is assumed the bacteria entered the animal via the injection site, but an explanation of the reason the rump of the animals swelled and became contaminated when injection was 'guaranteed' to be in the neck by the farmer goes unexplained.

References

1. Collins MD, Falsen E, Foster G, Monasterio LR, Dominguez L, Fernandez-Garazabal JF. *Helcococcus ovis* sp. nov., a gram-positive organism from sheep. *Int J Syst Bacteriol.* 1999;49:1429–32.
2. Post KW, Rushton SD, Billington SJ. Valvular endocarditis associated with *Helcococcus ovis* infection in a bovine. *J Vet Diagn Invest.* 2003;15:473–5.
3. Locatelli C, Scaccabarozzi L, Pisoni G, Bronzo V, Casula A, Testa F, *et al.* Helcococcus kunzii and *Helcococcus ovis* isolated in dairy cows with puerperal metritis. *J Gen Appl Microbiol.* 2013;59:371–4.
4. AHVLA disease surveillance report. Bovine abortions caused by *Helcococcus ovis*. *Vet Rec.* 2014;175:38–41.
5. Mao L, Chen Z, Lu Y, Yu J, Zhou Y, Lin Q, *et al. Helcococcus ovis* in a patient with an artificial eye: a case report and literature review. *BMC Infect Dis*. 2018;18:401.
6. Flock&Herd website www.flockandherd.net.au .*Helcococcus ovis* isolated from suppurative arthritis in a lamb. August 2020 edition.

The power of vaccination Blackleg/Malignant Oedema case study

Cosmin Susa (BVSc, DVM, PGDipVCS), Hunterville Veterinary Club

In July 2021, 9 R1 Speckle Park beef bulls and steers died suddenly over a 24 hour period 5 days after being yarded for drenching (pour-on) and castration. The cattle had been bought from the South Island (in February) for finishing and had reportedly been previously vaccinated twice for clostridial disease prior to transport. Both castrated and uncastrated cattle were dead.

On post-mortem examination of multiple cattle the subcutaneous tissue on the ventral abdomen was oedematous and yellow and extended into underlying muscle in both castrated and non-castrated animals.

Clinically Blackleg was suspected, and post-mortem samples were submitted for culture and histology to Gribbles Veterinary Laboratory in Palmerston North.

Histologically the lesions were more consistent with Malignant Oedema with the subcutaneous tissue and fascia primarily affected. Anaerobic culture failed to isolate any *Clostridia*. Sacrificial euthanasia was recommended for further testing.

All remaining cattle were treated with high dose penicillin and vaccinated for clostridial disease (Covexin 10®, MSD Animal Health, A9028).

Another 5 calves died over the next 3 days but there were no more deaths from 5 days after vaccination.

One bull was lame with a markedly swollen upper right hind limb but was systemically well. The decision was made to euthanise this animal and submit further samples for histology and culture.

On post-mortem examination there were multiple areas of yellow, oedematous subcutaneous tissue on the ventrum, poll, distal limbs and groin.

The proximal hindlimb muscles were markedly swollen, darkly discoloured, and haemorrhagic.

Histologically there were similar oedematous changes to the first post-mortem, but no Clostridia were identified. There were more acute and chronic lesions involving the hindlimb muscles. Anaerobic culture was unrewarding from the affected muscle.

These cases are most consistent with Malignant Oedema but Blackleg could also be involved which would explain the sudden death of uncastrated cattle and the changes in the thigh muscles of the euthanised bull.

With the support from MSD Animal Health, tissues from both cattle were sent to UC Davis in the USA for immunohistochemistry to help identify the Clostridial species involved. The results showed Bull 1 was positive for *C.sordellii* and Bull 2 was positive for both *C.sordellii* and *C.chauvoei*. These additional findings confirmed Malignant Oedema in Bull 1 and Blackleg and Malignant Oedema in Bull 2.

Although Malignant Oedema and Blackleg are both clostridial diseases, the disease pathogenesis and usually the pathogens involved are different although *Clostridium chauvoei* can cause both.

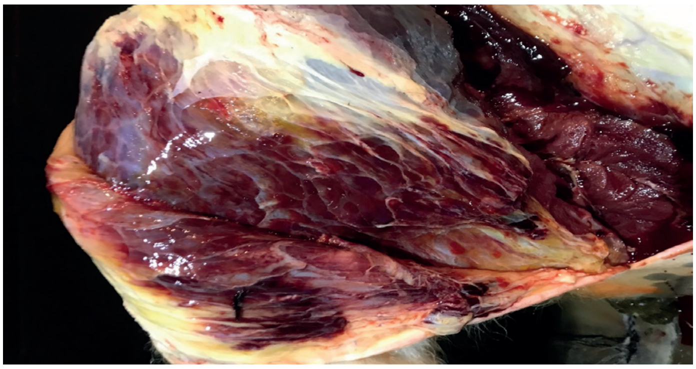
Malignant Oedema usually occurs secondary to soil contamination of wounds, in this case castration wounds, whereas Blackleg is caused by sporulation of pre-existing clostridial spores in the muscle due to creation of a localised anaerobic environment (usually blunt trauma e.g., yarding).

Malignant Oedema has been associated with *Clostridium septicum, sordellii, chauvoei, novyi* and *perfringens*. Blackleg is only caused by *Clostridium chauvoei*. It is worth noting that Malignant Oedema is the only disease in NZ ruminants where *Clostridium sordellii* has been confirmed to cause clinical disease.

Vaccination in the face of an outbreak was very effective in reducing the morbidity and mortality rate although deaths continued for a number of days until there was an adequate immune response. A booster vaccine was recommended 2-4 weeks later (on label it is 4-6 weeks apart) even if mortalities had ceased.

Live clinically affected stock is rarely seen with both Malignant Oedema or Blackleg and very rarely saved but in some cases the high dose penicillin was effective in preventing mortality, but clinical lesions will still persist in affected tissue reducing carcass value.

Vaccination for clostridial disease is usually extremely effective in prevention when label directions are followed, and boosters are given. In this case, disease could have been due to lack of vaccination, improperly handled/ administered vaccine, or less likely vaccine lack of efficacy. Overall mortality for this case was 23% of the mob.



Therefore, this case is unusual, and the previously reported vaccination was not able to be confirmed through correspondence with the previous owner.

Thank you to Geoff Orbell from Gribbles, Palmerston North and Amanda Kilby from MSD Animal Health for the help and support with this interesting case.

Yersiniosis in Aotearoa: concerning, cryptic and costly

Jackie Benschop, Molecular Epidemiology and Public Health Laboratory, Tawharau Ora, School of Veterinary Science, Massey University.

Yersiniosis is a debilitating zoonotic disease that is increasing in Aotearoa New Zealand. There were 1,411 notifications of yersiniosis in humans in 2021, an approximate doubling of notifications in 2017. Most cases in humans are due to *Y. enterocolitica* but a large outbreak in 2014 (300 cases over two months) was associated with *Y. pseudotuberculosis*. The source of this outbreak was not identified. Generally, people present with fever and bloody diarrhoea, 15% of cases are hospitalised, and the abdominal pain may mimic appendicitis necessitating exploratory laparotomy with post-surgical recovery and potential for complications. Adding to the human health burden, chronic sequelae, such as reactive arthritis can occur. Based on routinely collected notification data hypothesised risk factors include consumption of food from retail premises and contact with farm animals, yet the reservoirs of infection remain cryptic. To elucidate risk factors an ESR-led case-control study begun in 2021, focusing on cases of yersiniosis notified to Canterbury, South Canterbury, and the West Coast District Health Boards.

Yersiniosis is also an important disease for agriculture in Aotearoa. In cattle the major pathogenic species is *Y. pseudotuberculosis* with *Y. enterocolitica* in second place. Bovine yersiniosis typically presents as diarrhoea and ill thrift due to enteritis, although it has been associated with sporadic cases of abortion and death. Surveillance by New Zealand Veterinary Pathology reports an increase in yersiniosis in cattle over the period 2010 to 2014. Veterinary laboratory submission data from 2011 to 2017 shows this upward trend is continuing: there were approximately 25 notifications per week affecting high value dairy stock over the 2016/2017 summer season. We performed a descriptive analysis of these data and interviewed experienced dairy cattle veterinarians to make a preliminary estimation of the impact on the New Zealand dairy industry.

We investigated the diversity in *Yersinia* via whole genome sequencing of 52 isolates sourced from over 1,500 samples collected in 2017. These were primarily from healthy and sick livestock but also from wildlife, horticultural soil, nematodes and water. In New Zealand, *Yersinia* are carried by a wide variety of hosts and host-specificity is not tightly linked to genomic clade. As *Yersinia* spp. are often commensal it is important to consider ruling out differential diagnoses and including samples for histopathology.

Note: this abstract is based on one prepared for the NZVA meeting 2019. I want to acknowledge the following co-authors of the work presented then Ian Bruce, Sue M. Cassells, Nigel P. French, Alesha K. Meehan, Anne C. Midwinter, David A. Wilkinson.

Footnote, written by MSD Animal Health

A6151 Yersiniavax® is a vaccine designed, manufactured and registered for use in DEER ONLY.

It's use in cattle is completely off label, and not recommended. Anaphylaxis and deaths have been reported after use in cattle. Should a veterinarian choose to recommend this to their own clients it is at their own risk and the use of consent forms is recommended.

Cohort study of salmonellosis outbreaks on dairy farms in New Zealand

Chris Compton EpiCentre, School of Veterinary Science, Massey University

Background

The Ministry of Primary Industries (MPI) Surveillance Early Aberration Reporting System (EARS) detected in 2021 a trend of an increasing number of diagnoses of salmonellosis in dairy cattle in the years following 2012, particularly of *Salmonella* Bovismorbificans, and the emergence of a novel serotype in NZ cattle, *Salmonella* Give. This prompted a response from MPI to commission the EpiCentre, Massey University, to undertake the National Salmonellosis Case-Control Study to investigate potential risk factors for this increase in diagnoses, and MSD Animal Health to undertake a series of roadshows around New Zealand to increase awareness of the situation to veterinarians.

Research aims and objectives

We aimed to address three research questions in this study. First, we aimed to describe the pattern of non-Brandenburg salmonellosis outbreaks affecting more than 5% of cows in a sample of NZ dairy herds recruited from the National Salmonellosis Case-Control Study currently undertaken by Massey University. Second, we aimed to investigate the associations between the occurrence of clinical salmonellosis and animal-level factors such as age and breed. Finally, we aimed to describe the impact of diagnosis and treatment for salmonellosis on the risk of culling or death and on milk production, the patterns of usage of registered veterinary medicines (RVM's) for treatment of cases and details of any herd vaccination programmes used.

The objective of this research is to provide farmers and veterinarians with information that will identify preventive animal health measures to control salmonellosis outbreaks.

Materials and methods

Study design

This was a retrospective cohort study (hereafter named as the 'Study') among dairy farms diagnosed with naturally occurring outbreaks of acute salmonellosis from which non-Brandenburg serotypes were isolated from clinical cases. Farmers enrolled in the National Salmonellosis Case-Control study that indicated they would be willing to be contacted again following their interview by Massey University researchers were contacted by e-mail and phone by the author of this report and invited to enrol in the current study.

We requested farmers provide researchers with access to herd demographic, production, animal treatment, mating, pregnancy testing and animal removal data via their MINDA LIVE, myHerd or InfoVet or Farm Source electronic Dairy Diary software, or paper records, for the study period of the 2021–2022 dairy season (1 June 2021 to 31 May 2022). A follow-up time at least as late as May 2022 was required to gather records for production, reproduction, culling and death for the full dairy season. Farmers that had completed interviews for the National Salmonellosis Case-Control Study were contacted between May and July 2022. Farmers that agreed to participate provided the researcher with third party access to their herd management software and animal health records for the 2021 - 2022 dairy season.

Data management and statistical analysis

The definition of a farm with an outbreak of salmonellosis is one or more mixed-age cows displaying clinical signs of acute onset of diarrhoea with symptoms of systemic illness, initially with a high fever (rectal temperature 40 to 41°C) that subsided with the onset of diarrhoea, with or without abortion (esp. heifers) or cow deaths over a 14 day period. Diarrhoea may be severe and accompanied occasionally by dysentery and tenesmus. The clinical diagnosis was confirmed with laboratory isolation of *Salmonella* spp. that were serotyped as non-Bovismorbificans.

Cows from the enrolled herds were included in the analysis if they were present in the herd on 1 June 2021, calved during the period 1 June 2021 to 31 Oct 2022 and were either not removed from the herd in the study period, or if they were removed, their date of removal was on or following the date of first diagnosis of salmonellosis in the herd. Cows were classified by age as 2 , 3 - 4, 5 - 6, or 7+ year old's, and by breed as Friesian or Jersey if ≥ 12/16 ths of that breed, or otherwise as Crossbred (XB). The date of recorded treatments for animal health conditions, including for salmonellosis, was assumed to be the same as the day of diagnosis, and are defined as the date of diagnosis of salmonellosis in this report. A fatality was associated with salmonellosis if it occurred within 21 days of the diagnosis in the same cow.

The enrolled herds and the cases of salmonellosis were described using standard statistical and graphical methods. The associations between the occurrence of treatment for clinical salmonellosis and animal-level factors such as age and breed were investigated using multivariable logistic regression models, with the herd of origin as a fixed effect. The effect of salmonellosis on risk of removal from the herd was investigated by Mantel-Haenszel analysis. Finally, the effect of salmonellosis on milk solids production was investigated with linear multivariable models using multiple imputed chained equations because of missing breeding values in the records of cows removed from the herds.

Approval to undertake this study was given by the Massey University Human Ethics Committee (Protocol Number SOA-21-67).

Results

The number of herds and cows aimed to enrol calculated by power analysis were 5 herds and 2,160 cows, respectively. Nine farmers were contacted by the author and invited to participate in the Study, and five farmers agreed to do so and provided third party access to their animal health records and herd management software.

Description of farms

A total of 5 herds and 1,981 cows were enrolled in the study (Table 1). One herd did not undertake herd testing and therefore did not contribute milk production records to the analysis.

Table 1: Description of study herds in a retrospective cohort study on the effect of salmonellosis outbreaks on health and production

Variable	Level/statistic	Value
Herd ID	1	363 (18%)
	2	305 (15%)
	3	436 (22%)
	4	574 (29%)
	5	303 (15%)
	Total	**1981 (100%)**
Age category	2	384 (19%)
	3-4	684 (35%)
	5-6	471 (24%)
	7+	442 (22%)
	Total	**1981 (100%)**
Breed category	F	1136 (57%)
	J	48 (2%)
	XB	797 (40%)
	Total	**1981 (100%)**
Lactation milk solids (kg)	Min / Max	0 / 738.0
	Med [IQR]	384.0 [323.0;438.0]
	Mean (std)	373.7 (99.6)
	N (NA)	1659 (322)

Description of disease diagnoses and fates of animals removed from study herds

The overall number and incidence of cows diagnosed with salmonellosis and other common disorders, together with the fate of all animals remove from the Study herds is summarised in Table 2.

Table 2: The number and incidence of salmonellosis and common disorders together with the number and incidence by fate of all cows removed from Study herds

Variable	Herd 1	Herd 2	Herd 3	Herd 4	Herd 5	Total
Salmonellosis	20 (5.5%)	5 (1.6%)	2 (0.5%)	40 (7.0%)	8 (2.6%)	75 (3.8%)
Mastitis	14 (3.9%)	38 (12.5%)	65 (14.9%)	66 (11.5%)	55 (18.2%)	238 (12.0%)
Lame	2 (0.6%)	0 (0%)	44 (10.1%)	5 (0.9%)	10 (3.3%)	61 (3.1%)
Died	3 (0.8%)	1 (0.3%)	8 (1.8%)	16 (2.8%)	4 (1.3%)	32 (1.6%)
Culled or sold	69 (19.0%)	22 (7.2%)	62 (14.2%)	113 (19.7%)	57 (18.8%)	323 (16.3%)
Carried over	0 (0%)	0 (0%)	0 (0%)	50 (8.7%)	0 (0%)	50 (2.5%)
Culled or sold or carried over	69 (19.0%)	22 (7.2%)	62 (14.2%)	163 (28.4%)	57 (18.8%)	373 (18.8%)

The overall incidence of clinical salmonellosis in the Study herds was 3.44% (range 0.46% to 6.97%).

Description of salmonellosis outbreaks

The calendar dates of case of salmonellosis on each farm varied between 07 September 2021 and 11 January 2022 (Figure 1), which were between 27 and 127 days after the planned start of calving dates for each herd.

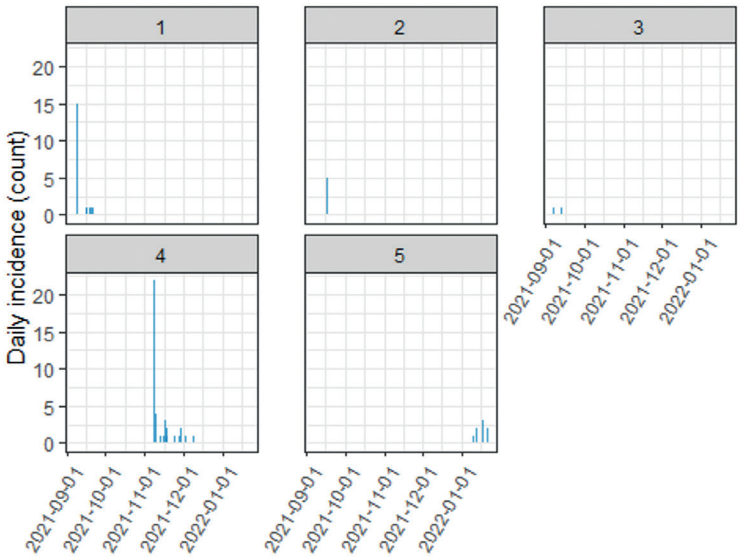


Figure 1: Epidemic curves of salmonellosis outbreaks of 5 dairy farms indicating the number of cases per day

The outbreaks were mainly characterised by the sudden appearance of a high proportion of the total number of cows affected with salmonellosis in a short period of time, followed by a decline in diagnosed cases over a few weeks. The mean duration of farm outbreak was 12 days (range 0 to 32 days).

Veterinary medicines used for treatment of cows with salmonellosis

The antibiotics used for treatment of cows with salmonellosis were either oxytetracycline in either long-acting ("Bivatop® (A6867)", 1-dose) or short-acting ("Engemycin® (A3308)", 3-dose) formulations, or the potentiated sulphonamide "Amphoprim® (A4730)" (3-doses, usually). Additionally, some cows were treated with non-steroidal anti-inflammatory formulations ("Metacam® (A7982)" or "KetoMax (A11031)"), and one farmer used Eprinex® Pour-On (A7191), an anthelmintic, without veterinary consultation.

Vaccination usage associated with the outbreaks

Vaccination of the whole herd with Salvexin®+B was undertaken on 4 farms, on average 1 day after the first case was treated (range = 0 to 3 days). A booster vaccination was given to all the cows in 1 herd 31 days after the first vaccination.

Associations between cow characteristics and diagnosis of salmonellosis

The odds of being diagnosed with salmonellosis were greater in cows aged 5 years and older compared with 2 year old cows, but was not associated with cow breed, and varied among herds. The probability of being diagnosed with salmonellosis of cows in Herd 1 was 2, 2.1, 7.3, 9.85 % for cows of age 2, 3 - 4, 5 - 7 and 7+ years, respectively.

Impact of salmonellosis on milk production and risk of removal from herds

Diagnosis with salmonellosis was significantly associated with reduced lactation milk solids production. Figure 2 displays the model coefficients to show that 2-year old Friesian cows in Herd 1, diagnosed with salmonellosis, without a diagnosed case of mastitis and with a breeding values for milk fat and protein of zero, were estimated to produce 24.77 kg less milk solids in the lactation compared with cows of the same characteristics but not diagnosed with salmonellosis (second bar from the bottom of the graph).

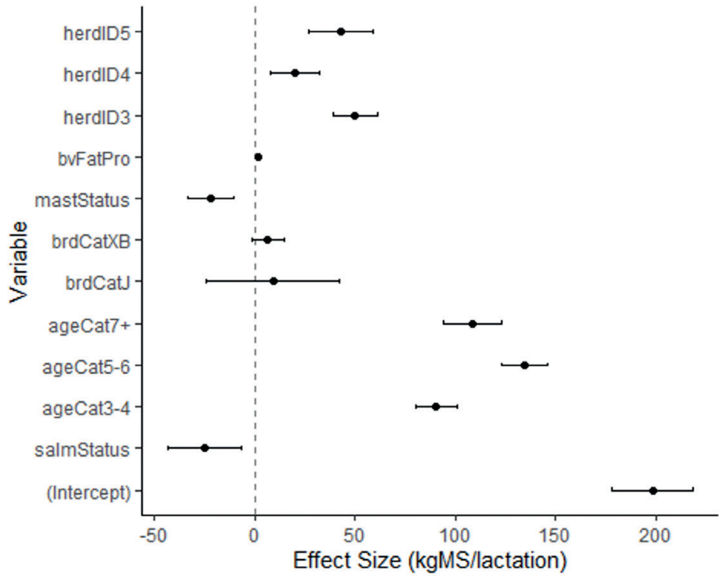


Figure 2: Forest plot of coefficients and their 0.95 confidence intervals from a model of effects of salmonellosis status (salmStatus), age category, breed category, mastitis status (mastStatus), breeding value for fat plus protein production and herd identity

The risk of removal from the herd because of culling or sale or being carried over for one season was greater in cows diagnosed with salmonellosis compared with those that had not been cases. The difference in risk of removal from the herd for cows with and without salmonellosis varied between herds (Table 3),and was greatest in Herd 4 in which the outbreak occurred over the herd mating period. The overall Mantel-Haenszel adjusted risk of removal from a herd in cows with salmonellosis was increased 1.36 -fold (95% confidence interval 0.96, 1.93) compared with cows that were not diagnosed with salmonellosis. The salmonellosis case fatality rate was 0% after accounting for the time between diagnosis and death.

Table 3: Count and incidence of cows removed from herds grouped by salmonellosis different fates at removal and by study herd

	Herd 1		Herd 2		Herd 3		Herd 4		Herd 5	
Fate at removal	**Negative**	**Positive**	**Negative**	**Positive**	**Negative**	**Positive**	**Negative**	**Positive**	**Negative**	**Positive**
Culled or sold	67 (20%)	2 (10%)	22 (7%)	0 (0%)	62 (14%)	0 (0%)	100 (19%)	13 (32%)	55 (19%)	2 (25%)
Carried over	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	44 (8%)	6 (15%)	0 (0%)	0 (0%)
Culled or sold or carried over	67 (20%)	2 (10%)	22 (7%)	0 (0%)	62 (14%)	0 (0%)	144 (27%)	19 (48%)	55 (19%)	2 (25%)

Discussion

Previous reports of salmonellosis outbreaks have mainly described overall measures of morbidity and mortality, and some even provided costs attributed to the outbreaks. This is the first detailed report of the pattern of non-Brandenburg Salmonella spp. outbreaks on N.Z dairy farms including the investigation of cow-level risk factors for disease and the impact on milk production and risk of removal from the herd.

The stage within lactation that salmonellosis outbreaks occurred in the study herds varied widely, and notably was not confined to the early calving period as has often been reported. This data demonstrates that the impact of salmonellosis outbreaks might be strongly associated with the stage of lactation when it occurs, as demonstrated by the greatest impact on removal from the herd occurring when the outbreak occurred during the breeding programme. This impact has not been clearly described previously and is important to consider in an economic analysis of a salmonellosis prevention programme as animal removal is very costly.

A novel finding in this study is that cows of 5-6 and 7+ years of age were at greater risk of salmonellosis in farms with outbreaks. The reason for this is unclear but should be considered for surveillance and biosecurity measures if a farm outbreak occurs. Additionally, the age distribution of salmonellosis cases would likely be impacting the reduced milk solids production recorded in this study from cows with the disease, as these age groups are the most productive in dairy herds.

Several limitations of this study should be considered when making inferences to the target population of N.Z dairy herds with salmonellosis outbreaks. First, the study herds were a convenience sample of farmers and could not be considered representative of the target population. Nevertheless, the morbidity rate and stage of lactation when the outbreaks occurred varied widely as might be expected to occur in the target population. Second, the target sample size of cows to enroll and expected number of affected cows were not enrolled, which meant that statistical power was reduced and effects truly existing were not discovered. However, the statistically significant associations were found between risk factors for and impacts of salmonellosis for the variables described in the study aims.

Despite these limitations, the author believes that this report provides useful data for vets and their farmers to consider when planning prevention or control programmes, based as they are on sound statistical analysis and clinical reasoning. This report provides the key information on the costs of salmonellosis outbreaks arising from veterinary fees, reduced milk production and increased risk of removal of affected cows from herds, all necessary for an economic analysis of prevention and control programmes.

Acknowledgements

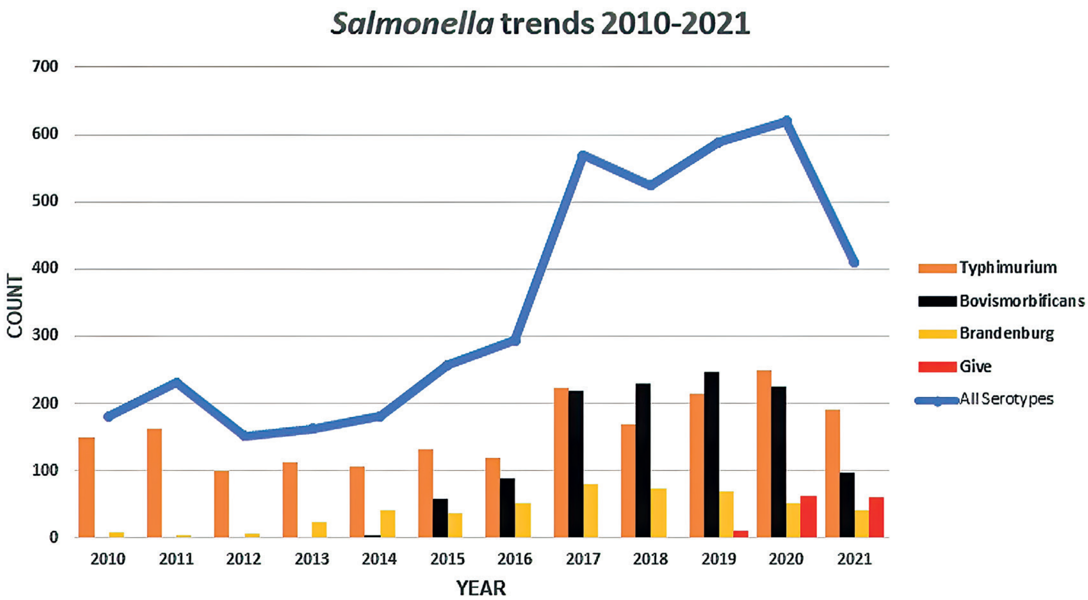
The author of this report thanks MPI (Paul Bingham and Jon Watts) who commissioned the National Case Control Study on Salmonellosis on dairy farms, and for supporting this Study. I also want to acknowledge the work of Nelly Marquetoux (EpiCentre, Massey) who assisted with data management.

Enteric Bovine Salmonella Sensitivities to Commonly Used Antibiotics in New Zealand

Jo Holter, MSD Animal Health

Salmonellosis is a significant disease of dairy cattle worldwide. A 2019 systematic review indicated that 9% of dairy cattle globally shed *Salmonella* in their faeces (Gutema, 2019). There are no recent comparable surveys in New Zealand but work in the 1960's recovered *Salmonella* from the faeces, mesenteric lymph nodes, spleen or intestine of up to 15% of dairy cows and bobby calves at slaughter (Nottingham & Urselmann 1961, Royal 1968). At that time, Typhimurium was by far the most prevalent serotype. From 2013-2021, reported *Salmonella* cases in cattle, each one representing a distinct outbreak, have been increasing until 2021. Most outbreaks in cattle are in dairy cattle and calves. In 2021 the cases of bovine Salmonellosis dropped 33% from the previous season, with cases of Bovismorbificans and Typhimurium dropping 58% and 23% respectively from the prior year (Surveillance, 2014-2021 & Jon Watts, MPI, personal comm. Figure 1). It is unclear why there has been a significant drop in cases, but factors such as decreased disease prevalence, increased disease awareness and preventative vaccination, under reporting due to the pandemic and staff shortages on farm may be contributing factors. Serotypes Typhimurium and Bovismorbificans (primarily enteric clinical disease) are distributed nationwide, while Brandenburg (primarily abortive clinical disease) is primarily restricted to the South Island, with sporadic cases isolated in the North Island. *Salmonella* Give is an emerging serotype with 2019, 2020 and 2021 outbreaks occurring in Waikato and more recently in the Bay of Plenty and Northland regions.

Figure 1: Bovine Salmonella Trends from MPI Passive Surveillance Data MPI, 2022, courtesy of Jon Watts



Bovismorbificans tends to cause high mortality outbreaks more commonly than Typhimurium: morbidity and mortality are reportedly up to 46% and 8%, respectively, in adult cattle, and up to 75% and 50%, respectively, in calves (Hulme-Moir, 2020). Bovismorbificans is a serogroup C *Salmonella*. Other serogroup C *Salmonellae* are known to readily induce prolonged carrier states, develop anti-microbial resistance, and cause septicaemia, and are of particular concern to the human medical community (Fuche, 2016).

Disease outbreaks can devastate dairy farms, indicating that *Salmonella* is endemic in that herd, which likely has carrier cattle and significant environmental contamination. Once *Salmonella* is diagnosed, it is not practical/possible to eliminate it from a farm. Clinical cases require antibiotic treatment and supportive care. Despite this, they may not recover productivity. Given the open nature of our dairy industry, it is likely that many farms which have never diagnosed Salmonellosis will however also have carrier cattle and/or environmental exposure, (at least for those serotypes which have been established nationwide), but have never had management triggers sufficient to precipitate an outbreak (Holschbach and Peek, 2018). An outbreak of enteric Salmonellosis causes, on average, 10% of cows to become sick, and approximately 1% to die (DCV, 2020). The estimated direct cost of an outbreak of this scale is $25,000-$30,000 for a 400-cow farm (Carter, 1983). Most cattle diagnosed with enteric salmonellosis tend to be treated with broad spectrum antibiotics and therefore in a typical outbreak, large numbers of cattle would be treated, and significant volumes of antibiotics used. Parental antibiotic use on farm has been associated with antibiotic resistance. Nobrega (2008) found that the risk of resistance increased with increasing antimicrobial daily doses of parental antibiotics. Therefore, parental antibiotic usage in outbreaks of Salmonella may increase the risk for antimicrobial resistance.

There is no annual monitoring of antimicrobial resistance to bacteria in domestic livestock species in New Zealand. The ESR does sporadically monitor antibiotic resistance to non-typhoidal and typhoidal *Salmonella* isolates in human, animal, food and from environmental sources. When ESR does monitor resistance only 20% of samples undergo culture and sensitivity, of which the majority of animal samples are bovine (based on submissions to ESR from the commercial veterinary laboratories reported in Surveillance, MPI). The last ESR report was published in 2016 and 4.5% of *Salmonella* in the animal/food/environmental category were resistant, of which 2.3% were resistant to Tetracyclines, 1.5% to sulphonamides and 1.5% were multidrug resistant. The next report is now pending and will report on the 2019 year. Preliminary data from the 2019 report which has been filtered for just animal/bovine cases indicates that approximately 10% of cases tested were resistant, 1% intermediate resistant and 2% were also resistant to more than one antibiotic (ESR, personal communication). Although this data should be viewed with caution until the official report is published, it may indicate a significant change in resistance rates and potentially coinciding with the peak of *Salmonella* Bovismorbificans cases.

Anecdotal reports of multidrug resistant *Salmonella* Bovismorbificans and the increase in *Salmonella* and *Salmonella* Bovismorbificans cases has prompted the need for more thorough surveillance of antimicrobial resistance in bovine *Salmonella* cases. Initially developed in conjunction with Gribbles Veterinary Pathology and now including the other veterinary commercial laboratories (IDEXX and SVS Laboratories) and Cognosco, bovine *Salmonella* isolates collected from July 2021 and typed at ESR have undergone sensitivity testing.

As of the 16th of September 2022, there have been 307 bovine *Salmonella* isolates cultured and sensitivities conducted (Figure 2). Of the main four serotypes seen in cattle, there were 149 cases of Typhimurium, 69 cases of Bovismorbificans, 40 cases of Give and 23 cases of Brandenburg (Figure 3). In most regions cases of Typhimurium are the predominate strain seen. Brandenburg is still primarily isolated to the South Island although one case was reported from the Waikato region. *Salmonella* Give continues to be primarily located in the Waikato region with one case reported in Northland and Bay of Plenty regions respectively.

Due to initial differences in standard culture panels from the laboratories, 291 isolates have had sensitivity testing to Tetracyclines and 290 isolates have had sensitivity testing to Trimethoprim Sulpha (TMS). Currently all laboratories are reporting isolates as either sensitive, intermediate and resistant based on CLSI Guidelines (disc diffusion). Some of the antibiotic sensitivities that are reported (e.g. Cephalothin) are not considered clinically effective against *Salmonella*, therefore are not discussed further. Treatment for enteric salmonellosis (excluding Dublin, an exotic serovar) include coverage with broad spectrum antibiotics e.g. oxytetracycline, trimethoprim sulpha or ceftiofur (Parkinson, 2010), with oxytetracycline and trimethoprim sulpha being the first and second line treatments respectively, therefore only Tetracycline and trimethoprim sulpha sensitives are reported here as these are the standard recommended treatments in New Zealand (NZVA, 2018).

Of the 291 isolates tested 5% were classed as intermediate and 5.5% were classed as resistant to tetracyclines. Overall, 10.5% of isolates were considered non-sensitive and 89.5% of isolates were sensitive to tetracyclines. When compared to the 2016 data from ESR there does appear to be an increase in resistant cases, however this should be viewed with caution as only 20% of isolates typed had sensitivity conducted. The preliminary data from the yet to be released ESR 2019 report appear to collaborate the apparent increase reported with the data presented in this report. Of the 16 resistant isolates, 5 were typed as Bovismorbificans and 11 typed as Typhimurium. Of the intermediate isolates, 2 were typed as Bovismorbificans, 1 Brandenburg, 1 Give, 1 Mississippi and 10 Typhimurium respectively.

Of the 290 isolates tested only 0.3% or 1/290 isolates were classed as resistant to trimethoprim sulpha. This was typed as *Salmonella* Bovismorbificans and was also resistant to Tetracycline but was sensitive to Enrofloxacin. This was the only isolate found to be resistant to both Tetracyclines and trimethoprim sulpha.

In a 2013 study from the northeastern United States monitoring antibiotic resistance to Salmonella isolates in dairy cattle found 44.3% were resistance to tetracycline, 42.3% of isolates were resistance to oxytetracycline and 6.6% were resistant to Trimethoprim sulfa (Cummings et al 2013). Similarly, a 2004 study from the southwestern United States also reported high levels of antibiotic resistance to *Salmonella* in dairy cattle, with 20.9% and 11.9% of isolates resistant to tetracyclines and trimethoprim sulfa respectively (Edrington et al 2004). A review of antibiotic resistance to Salmonella in the United States examined 16 studies from within the United States and 5 from outside the United States and found that resistance to tetracyclines ranged from 5.1 to 85% of isolates tested with the prevalence tending to be higher in cattle that were described as being clinically sick (Alexander et al 2009). Overall, the resistance to commonly used antibiotics in New Zealand is low compared to reported rates overseas but do appear to be increasing. Multi-resistant Salmonella remain rare despite an increase in Bovismorbificans isolates. Therefore, further ongoing surveillance will be important to monitor antimicrobial resistance to Salmonella in cattle in New Zealand.

As of September 2022, the project is ongoing with support from MPI, Gribbles Veterinary Pathology, IDEXX, SVS Laboratories and Cognosco.

References:

DCV Executive. (2020). Salmonella Outbreaks in Dairy Herds. HoofPrint. 38:1. p 27

Ministry for Primary Industries. (2021). Surveillance Annual Report for 2021.

F.D. Gutema, G. E. Agga, R.D. Abdi, L.DeZutter, L. Duchateau, S. Gabriel. (2019). Prevalence and Serotype Diversity of Salmonella in Apparently Healthy Cattle: Systematic Review and Meta-Analysis of Published Studies, 2000–2017. Frontiers of Veterinary Medicine. 6:102.

NZVA. (2018). Antimicrobial Use Guidelines for Dairy. Version 6.0

Hoffmann M, Pettengill JB, Gonzalez-Escalona N, Miller J, Ayers SL, Zhao S, Allard MW, McDermott PF, Brown EW and Monday SR (2017) Comparative Sequence Analysis of Multidrug-Resistant IncA/C Plasmids from Salmonella enterica. Front. Microbiol. 8:1459. doi: 10.3389/fmicb.2017.01459

Holschbach, C.L. & Peek, S.F. (2018). Salmonella in Dairy Cattle. Veterinary Clinics of North America. 34:133-154.

Hulme-Moir, L. (2020). Emergence of Salmonella Bovismorbificans in New Zealand. Grazing Gazetter: Newsletter of the Society of Sheep and Beef Cattle Veterinarians of the NZVA. 58:16-17.

Stevenson, M.A. et al (2016), A case-control study to identify risk factors for acute Salmonellosis in New Zealand dairy herds, 2011-2012. Epidemiology of Infectious Disease. 144:2154-2164.

Fuche FJ, Sow O, Simon R, Tennant SM (2016). Salmonella serogroup C: current status of vaccines and why they are needed. Clin Vaccine Immunol 23:737–745. doi:10.1128/CVI.00243-16.

T.J. Parkinson, J.J. Vermunt, J. Malmo (2010) Salmonella, Chapter 4: Diseases Causing Diarrhoea, Diseases of Cattle of Australasia. The New Zealand Veterinary Association Foundation for Continuing Education (VetLearn).

Trott, D & Cobbold, R. (2009). Multi Drug Resistant Salmonella: Why is it Yet to Emerge in Australia? Proceedings of the Australian Veterinary Association (AVA) Annual Conferences, 2009 AVA Annual Conference, Cattle/Industry Stream.

P. M. Nottingham & A. J. Urselmann (1961) Salmonella infection in calves and other animals, New Zealand Journal of Agricultural Research, 4:5-6, 449-460, DOI: 10.1080/00288233.1961.10431602

Alexander et al (2009) Antimicrobial resistant Salmonella in dairy cattle in the USA. Vet Res Commun. 33(3):191-209. doi: 10.1007/s11259-008-9170-7.

Edrington et al (2004) Antimicrobial resistance and serotype prevalence of Salmonella isolated from dairy cattle in the southwestern United States. 10(1):51-6. doi: 10.1089/107662904323047808

Cummings et al (2013) Antimicrobial resistance trends among Salmonella isolates obtained from dairy cattle in the northeastern United States, 2004-2011, Apr;10(4):353-61. doi: 10.1089/fpd.2012.1285.

Nobrega et al (2018) Antimicrobial resistance in non-aureus staphylococci isolated from milk is associated with systemic but not intramammary administration of antimicrobials in dairy cattle. J. Dairy Sci, 101:7425–7436, doi.org/10.3168/jds.2018-14540

Figure 2: Salmonella Isolate Count By Serotype And Region (n=307)

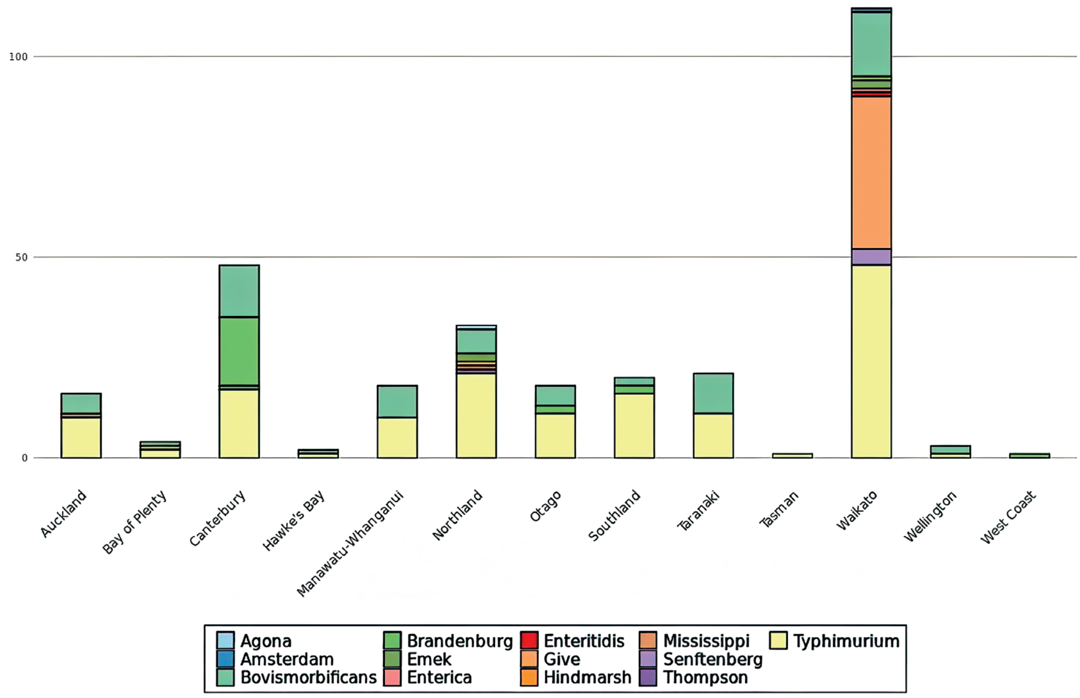


Figure 3: Main Bovine Salmonella Serotype Count By Region (n=283)

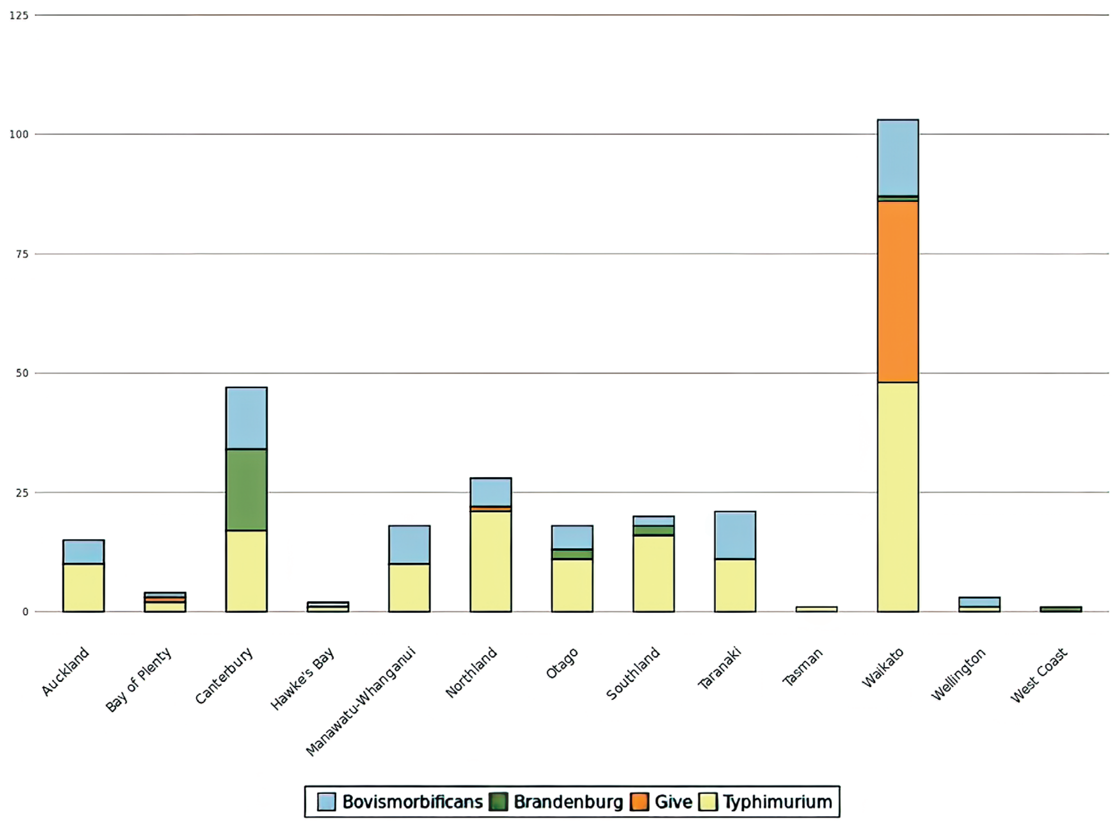


Figure 4: Bovine Salmonella Tetracycline Sensitivities By Serotype (n=291)

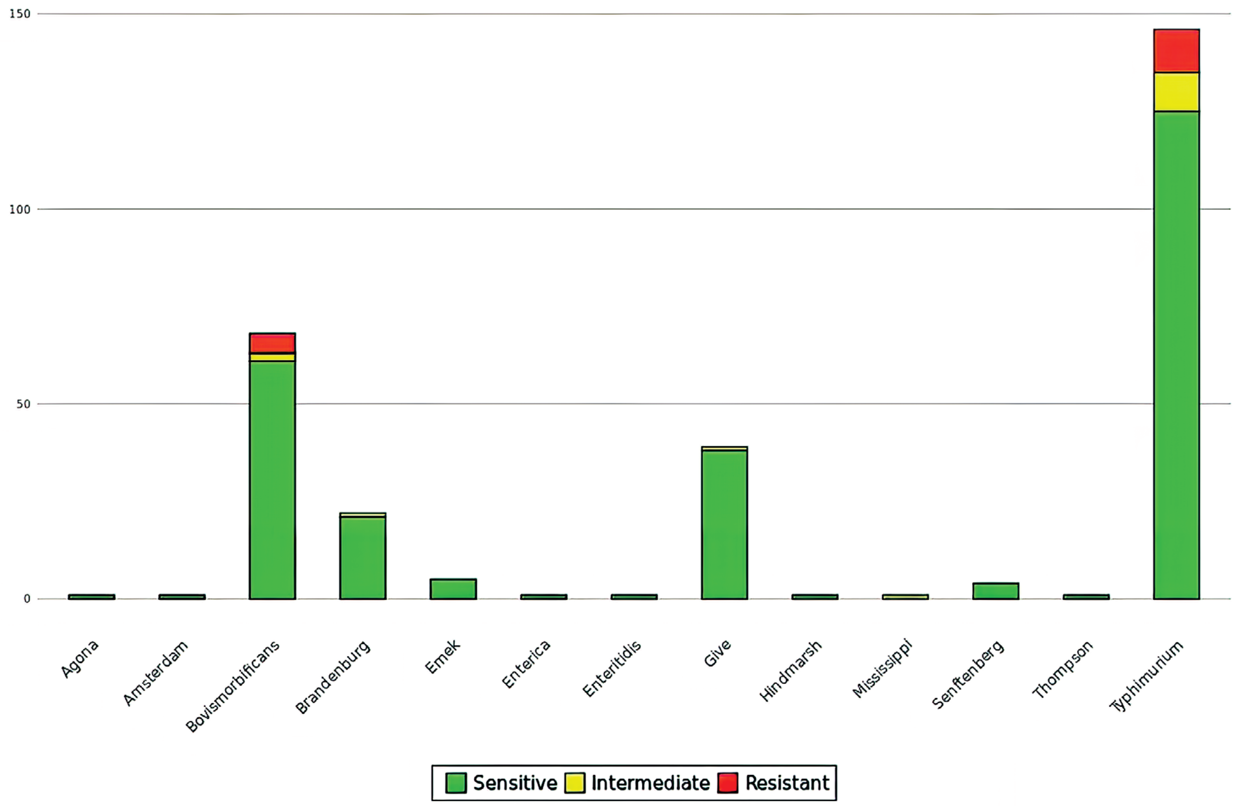


Figure 5: Bovine Salmonella Tetracycline Sensitivities n = 291

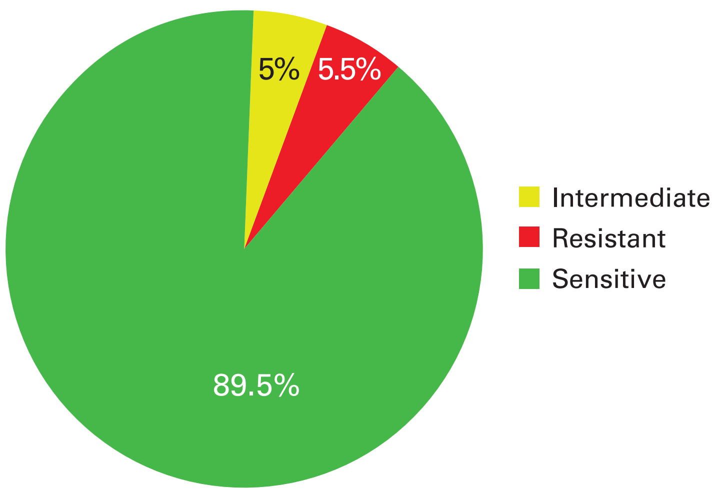


Figure 6: Salmonella Sensitivities to TMS by Serotype (n=290)

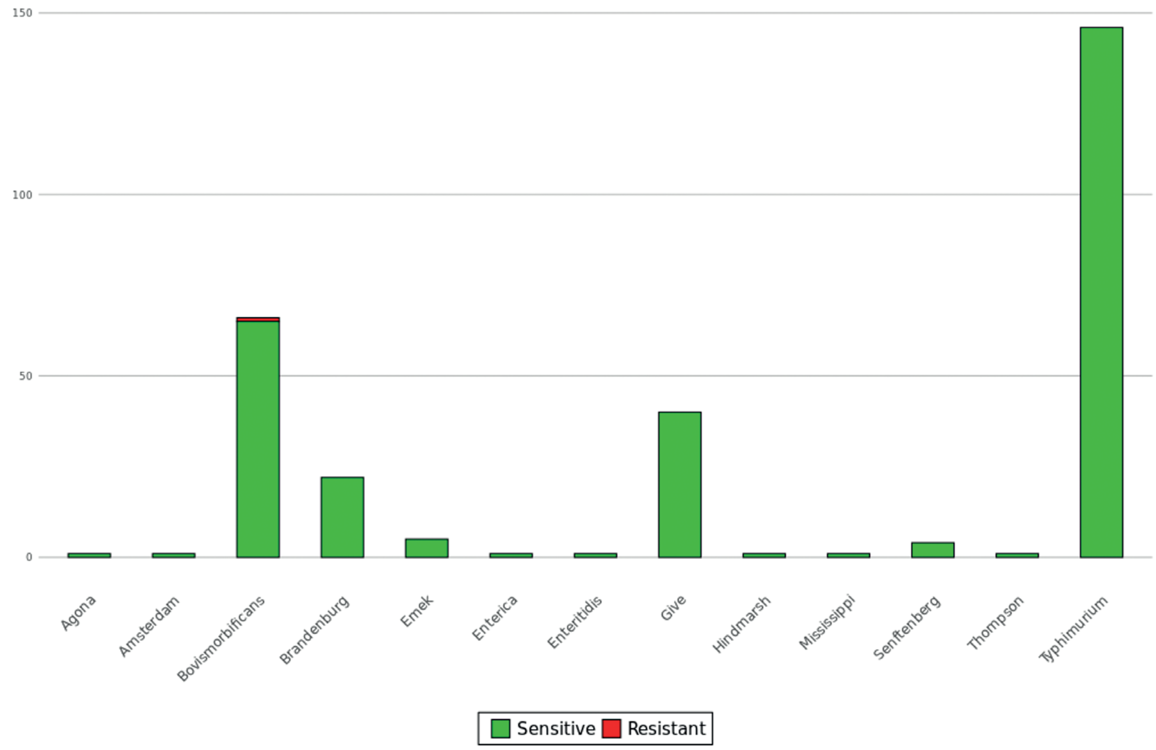
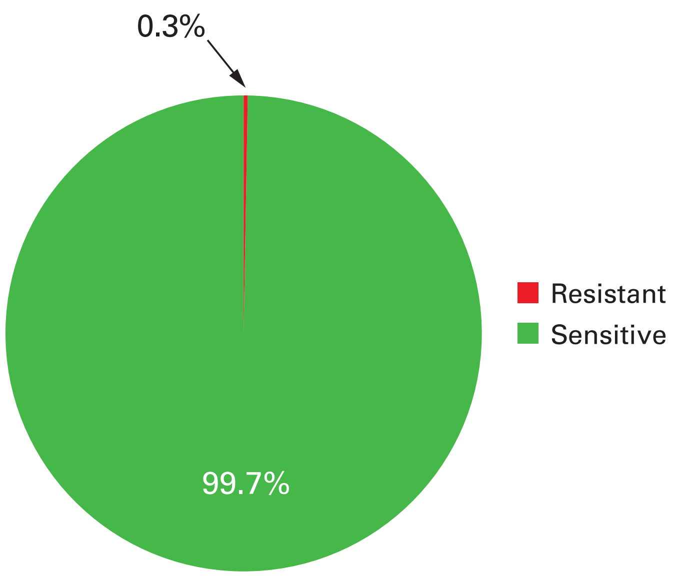


Figure 7: Bovine Salmonella TMS Sensitivities n = 290



Potential of ESBL-producing *Escherichia coli* selection in bovine faeces after intramammary administration of first generation cephalosporins using in vitro experiments

David C. Speksnijder[1,2], Nonke E. M. Hopman[1] , Nina E. Kusters[1] , ArjenTimmerman[1] , Jantijn M. Swinkels[3] , Pleun A. A. Penterman[4], Volker Krömker[5] , Andrew J. Bradley[6,7], Nadine Botteldoorn[8] , Ronette Gehring[9] & Aldert L. Zomer[1]

[1] Faculty of Veterinary Medicine, Utrecht University, The Netherlands.

[2] University Farm Animal Clinic, The Netherlands.

[3] Global Ruminant Business Unit, MSD Animal Health, The Netherlands.

[4] MSD Animal Health, The Netherlands.

[5] University of Copenhagen, Denmark.

[6] Quality Milk Management Services Ltd, UK.

[7] University of Nottingham, UK.

[8] Animal Health Care Flanders, Belgium.

[9] Institute for Risk Assessment Sciences, Utrecht University, The Netherlands

Introduction

Given the worldwide increase of antimicrobial resistance (AMR), efforts have been made in both human and veterinary medicine to prevent AMR selection and spread[1,2]. From a One Health perspective, the selection and spread of extended-spectrum β-lactamase (ESBL)-producing Enterobacteriaceae (ESBL-pE) within animal production systems and potential spillover to humans is a major concern[3]. When trying to curb AMR, it is important to eliminate inappropriate antimicrobial use (AMU).

The use of beta-lactam antibiotics, especially the use of extended-spectrum cephalosporins, in (food producing) animals is regarded as a risk factor for the emergence and spread of ESBLpE[4]. Therefore, the 1st–2nd and 3rd–5th generations cephalosporins are classified by the World Health Organisation (WHO) as Highly Important and Highest Priority Critically Important Antimicrobials for human medicine respectively. These antimicrobials should accordingly be used rationally and restrictively in veterinary medicine[5].

The selection and spread of antimicrobial-resistant Enterobacteriaceae occurs mainly in the gastrointestinal tract (GIT). There is limited evidence that the use of 1st and 2nd generation cephalosporins selects for ESBL-pE in the bovine GIT or in manure slurry, which could be a major route for transmission of ESBL-pE. First-generation cephalosporins like cephapirin (CP) and cefalonium (CL) are extensively used worldwide to treat and prevent intramammary infections in dairy cattle[6].

This raises the question whether 1st generation cephalosporins applied intramammary can select for ESBL-pE in the GIT of treated dairy cows, or in the environment once they are excreted via faeces or urine or discarded via waste milk in the slurry pit. The aim of this study was to gather scientific evidence on the risks for selection and spread of AMR after intramammary treatment of (sub)clinical mastitis in dairy cows with CP and CL and the associated ESBL selection in the bovine gut and in manure slurry, by using in vitro selection experiments.

Material and methods

Literature search

In November and December 2019, a non-systematic scientific literature search was conducted. Besides, a search was performed in publicly available scientific reports from authorizing institutes (i.e.European Medicines Agency) and monographs from veterinary pharmacology organizations, to gather information on pharmacokinetics and excretion routes of CP and CL after intramammary application in dairy cows. Scientific literature was searched for using Pubmed Central and Google Scholar using search strings “cephapirin”, “cefalonium”, “intramammary”, “dairy” and “pharmacokinetics”. Additionally, scientific information was searched for on usual intramammary treatment incidences and on faeces and manure slurry production of dairy cows. Based on these findings, calculations were made to estimate the maximum concentrations of CP and CL in the bovine GIT and in manure slurry.

Competition assays

Institutions from four different European countries (the United Kingdom, Belgium, the Netherlands, and Germany) were participating in the study. Between March and June 2019, three ESBL producing *Escherichia coli* isolates and three non-ESBL producing *E. coli* isolates were selected isolated from (bulk) milk, feces or bedding from three different dairy farms per country and sent to the laboratory of Utrecht University, The Netherlands. *E. coli* isolates able to grow on MacConkey agar plates containing 0.25 µg/ml CTX (Cefotaxime) (the ECOFF for CTX) were considered ESBL-producers (mic.eucast.org/search/). For each country, three sets of competition assays to study the potential effects of intramammary applied CP and CL to select for ESBL *E. coli* producers were performed.

Experiment 1 in enriched medium.

Per country, one colony from each of the three ESBL and three non-ESBL *E. coli* producer strains were dispensed in tubes containing 3 ml Lysogeny Broth (LB) medium (Oxoid, Tritium, The Netherlands) and incubated overnight in a shaking incubator at 37 °C. The next day, the ESBL and non-ESBL overnight cultures were mixed in a 1:3 ratio. Three µl of this mixture was added to nine different tubes containing 3 ml of LB medium. Additionally, antibiotics were added to seven of the nine tubes (0.08, 0.8 and 8.0 µg/ml of CP; 0.04, 0.4 and 4.0 µg/ml of CL; 0.25 µg/ml CTX (positive control)). Two tubes containing no antibiotics were used as negative controls.

Experiment 2 in fecal fermentations

Fresh fecal samples collected from 10 different healthy cows from three different farms (at least three cows per farm) from each of the four countries were transported under refrigeration (2–8 °C) to the laboratory of Utrecht University and arrived within 24 h after sampling. Per country, fecal samples were mixed to create one pooled fecal sample. Overnight cultures of the ESBL and non-ESBL *E. coli* strains (*E. coli* strains per country of origin) on blood agar plates were used. Equal parts of these McFarland suspensions from both the ESBL and non-ESBL *E. coli* strains were mixed in a fresh tube creating an 1:1 ESBL and non-ESBL mixture. This mixture was used to spike the nine fecal samples (per individual country) with± 107 CFU/gram, based on previous research. Just like in experiment 1, antibiotics were added to seven of the nine tubes (0.08, 0.8 and 8.0 µg/ml of CP; 0.04, 0.4 and 4.0 µg/ml of CL; 0.25 µg/ml CTX (positive control)). Two tubes containing no antibiotics were used as negative control.

Experiment 3 in manure slurry fermentations

Per country, from one randomly chosen dairy farm, a sample from the manure pit was taken. This sample was cooled and transported to the laboratory of Utrecht University within 24 h. The same procedure as for the pooled fecal samples in experiment [2] was followed, except for the temperature, that was kept at 17 °C. The circumstances in a manure slurry pit are different compared to the circumstances in the intestinal lumen. The temperature is much lower, around 15 °C, maximum in summer around 18–19 °C, under Western European conditions, and it contains both urine and feces, wastewater and sometimes waste milk.

Metagenomic sequencing.

To investigate the selection on ESBL production, DNA was isolated from 1 ml of feces and 1 ml of manure slurry of all nine tubes after experiment 2 and 3, using the protocol as described for the EFFORT-project. The sequence depth of each resistance gene was adjusted for sequencing depth to depth per gigabase. Resistance classes were obtained from the Resfinder database and hits to each resistance gene were summed per antimicrobial class.

Results

Literature

The literature based calculated maximum concentration of CP in feces after intramammary application is 0.114 µg/ml faeces, and for CL is 0.289 µg/ml faeces. The literature based calculated maximum concentration of CP in manure slurry is 0.023 µg/ml slurry, and for CL is 0.029 µg/ml slurry.

Experiment 1, 2, and 3

Figure 1, 2 and 3 summarise the findings of Experiment 1, 2 and 3, respectively, suggesting the Minimum Selective Concentration is > 0.8 µg/ml for Cefapirin (CP) and > 0.4 µg/ml for Cefalonium (CL). Such concentrations are higher than the estimated concentrations derived from the literature.

FIGURE 1. Average and standard deviation of the percentage of CTX resistance of *E. coli* in rich media experiments at different concentrations of CP, CL and CTX.

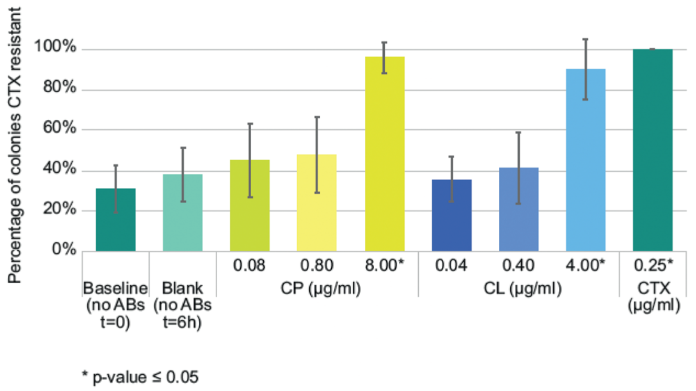


FIGURE 2. Average and standard deviation of the percentage of CTX resistance in fecal fermentation experiments at different concentrations of CP, CL and CTX.

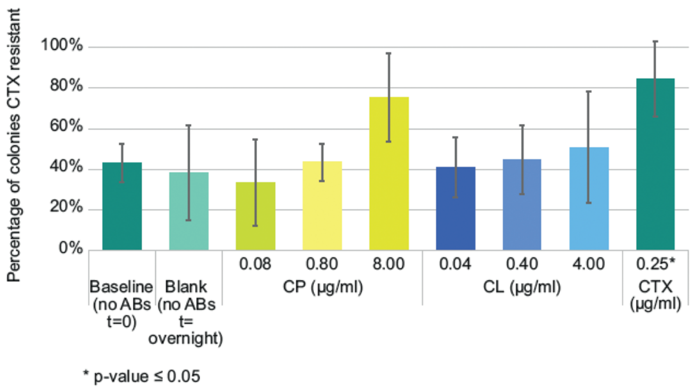
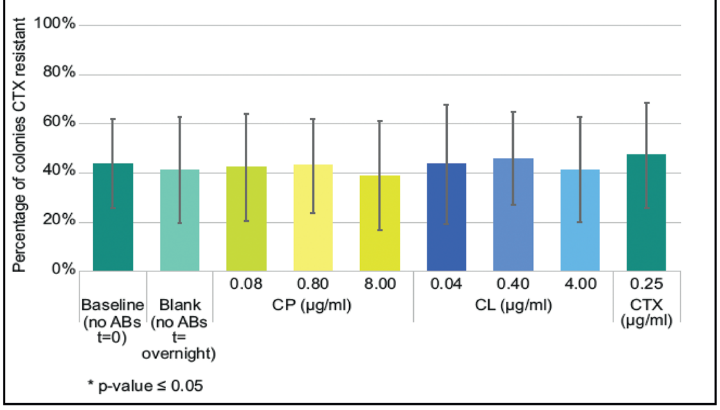


FIGURE 3. Average and standard deviation of the percentage of CTX resistance in manure slurry experiments at different concentrations of CP, CL and CTX.



The results of metagenomic sequencing confirm the results from experiment 1, 2 and 3.

Discussion

In our assumptions, we took the conservative approach (calculated with the highest possible concentrations of CP and CL in the gut and in manure) to minimize the risk of drawing the wrong conclusion that intramammary applied CP and CL do not select for ESBL-producing *E. coli* in the bovine gut. Our conservative approach related to pharmacokinetics or manure production might overestimate the calculated concentrations of CP and CL in faeces and manure slurry in our study. On the other hand, we used 24 h average concentrations and not peak concentrations that may occur for a few hours, although literature shows peak concentrations will not be very high either[7]. Also, the literature shows data of pharmacokinetics of healthy animals, but to which extend this represents diseased animals is not known.

Although the outcome of this in vitro study indicated the risk for AMR in the gut after treatment is low, we did not look at postpartum residues in colostrum after dry cow therapy going into the gut of the calf. Such studies are therefore warranted in the future.

Conclusion

In our study we found no evidence supporting selection of ESBL-producing *E. coli* in bovine feces or in manure slurry after intramammary use of commercial CP or CL containing products, using laboratory competition assays based on existing literature on pharmacodynamics and pharmacokinetics. The calculated maximum concentrations of CP and CL in the bovine gut and in manure slurry after intramammary administration will be substantially lower than the found minimum selective concentrations of CP and CL found to select for ESBL-producing *E. coli* in these environments. These results suggest that intramammary use of 1st generation cephalosporins in dairy cows do not contribute for the selection of ESBL producing bacteria, however confirmation with field studies are of paramount importance in the near future.

This study is accepted for publication in June 2022; www.nature.com/scientificreports/

References

1. Tang, K. L. et al., Restricting the use of antibiotics in food-producing animals and its associations with antibiotic resistance in food-producing animals and human beings: A systematic review and meta-analysis. Lancet Planet Health 1, e316–e327 (2017).
2. World Health Organization. Global action plan on antimicrobial resistance (2015).
3. Madec, J., Haenni, M., Nordmann, P. & Poirel, L. Extended-spectrum βlactamase/AmpC-and carbapenemase-producing Enterobacteriaceae in animals: A threat for humans? Clin. Microbiol. Infect. 23, 826–833 (2017).
4. Seiffert, S. N., Hilty, M., Perreten, V. & Endimiani, A. Extended-spectrum cephalosporin-resistant Gram-negative organisms in livestock: An emerging problem for human health? Drug Resist. Updates 16, 22–45 (2013).
5. World Health Organization. No title. WHO list of critically important antimicrobials for human medicine (WHO CIA list) (2019)
6. Brunton, L. A., Duncan, D., Coldham, N. G., Snow, L. C. & Jones, J. R. A survey of antimicrobial usage on dairy farms and waste milk feeding practices in England and Wales. Vet. Rec. 171, 296 (2012).
7. The European agency for the evaluation of medicinal products, veterinary medicines and information technology. Cefapirin Summary Report 2 (2001).

ShutOut®: Easy to use while matching performance

Rico Nortje, Rangiora Vet Centre & Penny Mehrtens, MSD Animal Health

ShutOut, a new internal teat sealant manufactured by MSD Animal Health has matched the performance of Teatseal® in a large-scale non-inferiority trial carried out on two Canterbury dairy farms during and after the 2021 dry period.

While the final results are being collated, initial trial data has shown no difference in performance between the products, which use the same formulation and active ingredient (bismuth subnitrate). The team administering the sealants, however, noticed the ShutOut was much easier to administer than Teatseal when the product is cold.

The trial was carried out on behalf of MSD by Rangiora Vet Centre (RVC) and led by large animal veterinarian Richard Nortje. The veterinary technician team administering the sealants was led by RVC's Kellie Grieve.

Richard Nortje says there is no other internal teat sealant with published non-inferiority trial work (compared with Teatseal) in lactating NZ dairy cows assessing key outcomes, including clinical and sub-clinical mastitis as well as product retention. While a number of generic internal teat sealants had been in the market for over 10 years, most lacked any robust trial work to back them up. This trial addressed these shortcomings, using two herds of about 1,000 cows each on two separate farms south of Rangiora.

The cows recruited for the trial were all lactating and in good body condition, with any lame animals excluded. Enrolled animals had somatic cell counts no more than 150,000 on their most recent herd test and no clinical mastitis throughout lactation.

Cows in each herd were randomly allocated into two equal-sized treatment groups, one receiving Teatseal and one receiving ShutOut. Neither group received antimicrobial dry cow therapy. The treatment types were identified using coloured leg bands and paint, and the two treatment groups ran together with no differences in management. Although the veterinary team were aware which treatment group was which, the herd owners / managers were not.

The trial monitored several important markers, including:

- incidence of dry period mastitis
- somatic cell counts in August and October (at the animal's first herd test)
- retention of internal teat sealant through the dry period to first milking after calving

Kellie Grieve noted there were "only a couple" of clinical dry period mastitis cases in the more than 1,100 trial cows, a low rate typically seen in in cows treated with an internal teat sealant.

She said treated cows were inspected in the paddock daily for up to 7 days after the sealant was applied to check for any isolated or sick cows, or swollen and red quarters – all signs that pathogens might have been introduced during the procedure. "We don't routinely do this with the herds we treat but we advise farmers to do paddock walks in the dry period to look out for signs of infection."

Data collected thus far shows no appreciable difference between the Teatseal and ShutOut cows in terms of dry period mastitis, somatic cell counts in the August and October herd tests, or retention of product in the teat canal.

A key difference, however, was ease of use, recorded anecdotally from the technician team. Kellie said the shorter and wider ShutOut syringes, whose design excludes the air bubble found in Teatseal syringes, made the job appreciably easier. And unlike Teatseal, the ShutOut syringe has a dual tip, which makes partial insertion easier and lowers the risk of teat canal damage.

She added that ShutOut seemed less prone than Teatseal to going hard in cold weather, and less in need of warming prior to use.

The application phase took place over several days in batches of a few hundred with younger cows treated first. Kellie said the weather was cold and very wet over this period. In fact the floods that devastated much of Canterbury in late May happened right in the middle of this phase. It didn't disrupt the work but as a precaution cows treated after the flooding were first checked by the rapid mastitis test (RMT) in case environmental mastitis had elevated their cell counts.


The Rangiora Vet Centre team at work applying the internal teat sealants in a trial mob.

Richard Nortje said that while the weather wasn't pleasant for the team, it was actually a bonus for the robustness of the trial. "A lot of product-related trial work is done using smaller sample sizes on research farms that may not reflect real-world conditions. This trial was done in very realistic commercial farm conditions with the cold wet weather you'll often find at drying off."

The trial has now been written up and is the process of being submitted for publication.

ShutOut was launched in 2022, and completes MSD's dry period mastitis management range. The ShutOut syringe was designed by MSD and is manufactured to a high standard in the same premises as many of MSD's other intramammary and injectable antimicrobials.

A randomized non-inferiority study evaluating the efficacy of two commercially available teat sealants in dairy cows.

Michelle P. Buckley[1], Tiago Tomazi±, Brian Miller[2], Jenna Bayne[1], Sandra Godden[3], Gustavo S. Silva[1], and Patrick J. Gorden[1].

Introduction

Subclinical intramammary infection acquired during the dry period can contribute to decreased production and milk quality (Gonçalves *et al.*, 2018). Failure of formation of a keratin plug early in the dry period leaves the teat at risk to acquire a new IMI (Dingwell *et al.*, 2004). Internal teat sealants (**ITS**) at dry-off have been shown to reduce the risk of acquiring a new IMI during the dry period (Vasquez *et al.*, 2018; Winder *et al.*, 2019).

Objective

The objective was to compare a new ITS (ShutOut, Merck Animal Health, Madison, NJ) to the first product on the market (Orbeseal [**ORB**], Zoetis, Parsippany, NJ) in a trial evaluating quarter-level intramammary infection (IMI) dynamics over the dry period and cow-level health events through 120 DIM.

Materials and methods

This study was conducted on six commercial dairy farms in Iowa (IA) and Minnesota (MN). Cows were cultured prior to dry-off and if eligible where treated with 500 mg cloxacillin benzathine (Orbenin DC, Merck & Co., Rahway, NJ,USA) and again after calving. Generalized linear mixed models were used to build univariable and multivariable models exploring the relationship between explanatory variables of interest and IMI at enrollment, IMI at 1-14 DIM, cured IMI risk, and new IMI risk (**NIMI**). A non-inferiority analysis evaluated the effect of ITS treatment on quarter-level dry period NIMI risk, with an a priori margin of non-inferiority established at 5%. The null hypothesis tested was that risk of NIMI for ShutOut was ≥+5% than the NIMI risk for ORB, thus asserting that ShutOut is inferior to ORB. Generalized linear mixed models were used to assess the risk of clinical mastitis, culling, and death before 120 DIM. Kaplan-Meier curves were developed to show the incidence of clinical mastitis, culling, and death. The effect of treatment on milk yield and $\log_e$ SCC were analyzed using repeated measures models.

Results

There were 418 cows enrolled in the ORB group and 404 in the ShutOut group. Risk difference estimates and 95% CI were determined from generalized linear mixed models in R. Final models included random intercepts for 'cow, herd, and cow within herd' for IMI at Dry Off (IMI at DO); 'cow and herd' for IMI at Post-Fresh (IMI at PF) and Cure IMI Risk; and 'cow' for New IMI Risk. Fixed-effect covariates included in the final model due to evidence for confounding when using the 10% change in estimate approach were: 'lactation' for IMI at DO and 'herd' for New IMI Risk. No fixed-effect covariates were included for IMI at PF and Cure IMI Risk. Adjusted dry period new IMI risk in the ORB group was 33.1% versus 31.5% for the ShutOut group. The risk difference (ShutOut minus ORB) was determined to be -1.6% (95% CI: -1.8, -1.37). A margin of noninferiority for risk difference of +5% was specified a priori. The lower and upper limits of the 2-sided 95% CI for the risk difference of new IMI risk were below the margin of inferiority, indicating that ShutOut was not inferior to Orbeseal.

Estimated least square means (plus 95% CI's) of test-day log SCC by month fresh during the first 100 d of lactation were compared using repeated measures analysis for cows receiving ORB vs ShutOut at dry-off (Graph A). There were no covariates included in the analysis. Repeated measures analysis did not determine an effect of treatment or treatment by month fresh interaction, but there was a difference detected between months fresh (P=0.008). Kaplan-Meier curves were developed to show the incidence of clinical mastitis (Graph B). No difference in mastitis incidence was detected (P=0.49).

TABLE 1. *Final generalized linear mixed models (logistic regression) estimating the effect of internal teat sealant group on quarter level dry period IMI dynamics.*

	Adjusted risk (%)	Risk Difference (%)	95% CI of Risk Difference	*P*-value
IMI at Dry Off				
Orbeseal	32.5			
ShutOut	29.9	-2.6	-2.8, -2.4	0.25
IMI at Post-Fresh				
Orbeseal	34.1			
ShutOut	31.5	-2.6	-2.81, -2.39	0.26
Cure IMI Risk				
Orbeseal	95.9			
ShutOut	96.7	0.8	0.007, 1.59	0.58
New IMI Risk				
Orbeseal	33.1			
ShutOut	31.5	-1.6	-1.8, -1.37	0.54

FIGURE 1. Non-inferiority hypothesis test for new intramammary risk difference between ShutOut and Orbeseal.

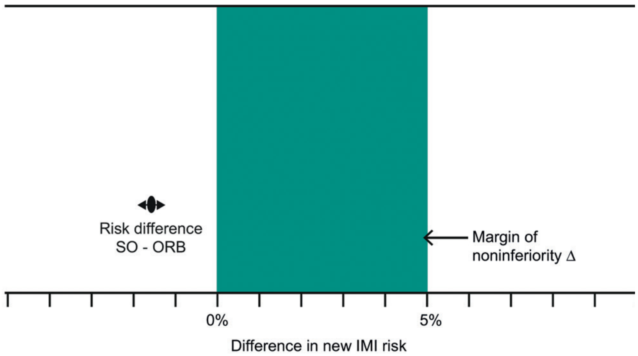
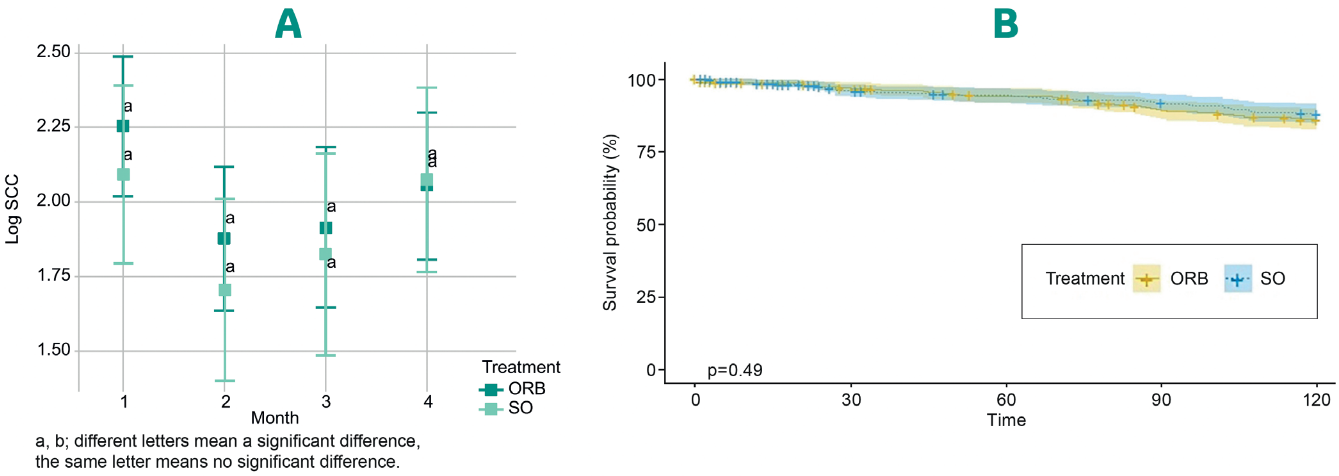


FIGURE 2. Least square mean estimates of log SCC by month fresh (A); B-Kaplan-Meier curve showing incidence of mastitis (B).



Authors' affiliation

1. Department of Veterinary Diagnostic and Production Animal Medicine, Iowa State University, Ames, IA.

2. Merck Animal Health, USA.

3. Department of Veterinary Population Medicine, University of Minnesota, St. Paul, MN, USA.

References

Gonçalves, J. L., Kamphuis, C., Martins, C., Barreiro, J. R., Tomazi, T., Gameiro, A. H., Hogeveen, H, and dos Santos, M. V. 2018. Bovine subclinical mastitis reduces milk yield and economic return. Live Sci, 210, 25-32. doi:https://doi.org/10.1016/j.livsci.2018.01.016.

Dingwell, R. T., Leslie, K. E., Schukken, K. E., Sargeant, Y. H., Timms, J. M., Duffield, L. L., Keefe, G. P., Kelton, D. F., Lissemore, K. D., and Conklin, J. 2004. Association of cow and quarter-level factors at drying-off with new intramammary infections during the dry period. Prev Vet Med, 63(1-2), 75-89. doi:http://doi.org/10.1016/j.prevmed.2004.01.012.

Vasquez, A. K., Nydam, D. V., Foditsch, C., Wieland, M., Lynch, R., Eicker, S., and Virkler, P. D. 2018. Use of a culture-independent on-farm algorithm to guide the use of selective dry-cow antibiotic therapy. J Dairy Sci, 101(6), 5345-5361. doi:https://doi.org/10.3168/jds.2017-13807.

Winder, C. B., Sargeant, J. M., Hu, D., Wang, C., Kelton, D. F., Leblanc, S. J., . . . O'Connor, A. M. 2019. Comparative efficacy of teat sealants given prepartum for prevention of intramammary infections and clinical mastitis: a systematic review and network meta-analysis. Anim Health Res Rev, 20(2), 182-198. doi:https://doi.org/10.1017/S1466252319000276.

Efficacy of a new teat sealant alone or combined with an existing dry cow antibiotic in a selective dry cow therapy program.

Swinkels, J. M.[1], A. Deterink[2], M. Holstege[2], A. Schmenger[3], G. D. Kempe[2], T. Bruggink[2], P. Penterman[4], C. Schepenzeel[2], A. Velthuis[2] and V. Kromker[5].

Introduction

Restriction on antibiotic use is putting pressure on farmers to switch from blanket dry cow therapy to selective dry cow therapy (SDCT), where cows are selected to be treated with antibiotics only when they have intramammary infections at dry off.

The use of teat sealants to prevent new infections are an important requisite to successfully implement SDCT programs. A new internal teat sealant was recently introduced on the market.

Objective

The objective of this study is to confirm the effect on udder health of the new teat sealant in a SDCT program both when used alone and when combined with antibiotic.

Materials and methods

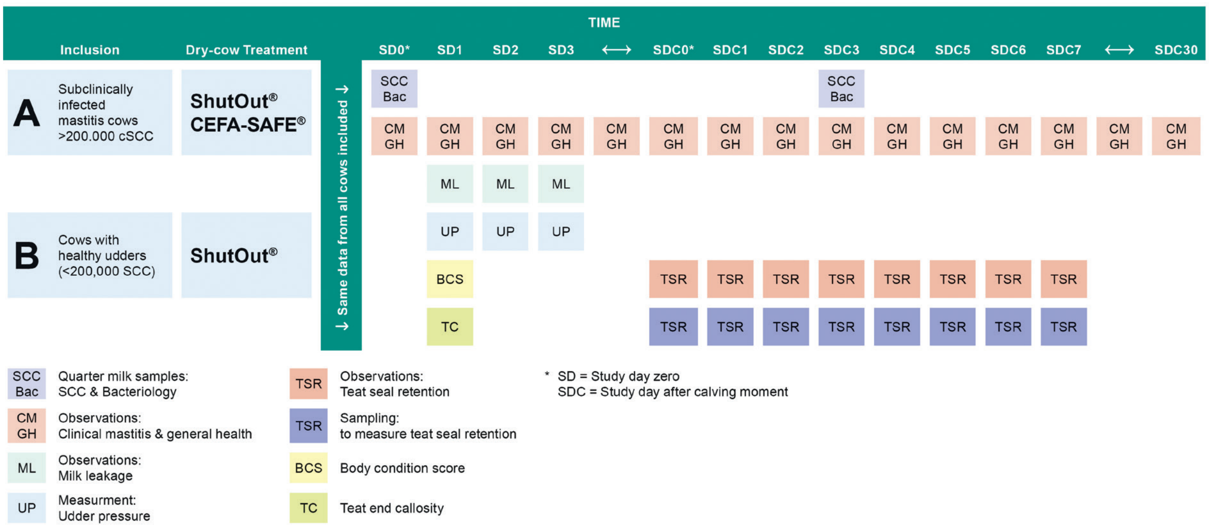
Seven herds were selected based on proximity to either Hannover, Germany (n=4), or Deventer, The Netherlands (n=3) were selected. 'High' SCC cows (Group A, > 200k cells/ml in at least 1 of the last 3 DHI tests before dry off , n=45) were treated intramammary with antibiotic (CEFA-SAFE®, MSD Animal Health), and an ITS (SHUTOUT®, MSD Animal Health) in all 4 quarters. 'Low' SCC cows (Group B) < 200k cells/ml in all 3 DHI teste before dry off, n=45) only received ITS in all 4 quarters. Milk samples for bacteriology and SCC were taken at dry off and at d3 postcalving to determine effects on udder health. General health, including clinical mastitis cases, were monitored from dry off until 30 days in the following lactation. Body condition score, teat-end callosity, milk production and leakage, and udder pressure were measured to determine their influence on udder health.

To determine cure and prevention rates a univariate and multivariate model was created to compare the 2 treatment groups.

Results

- Figure 1 shows the study design for the 2 different cow level treatment Group A and B from dry off (SD0) until 30 days postcalving (SDC30).

FIGURE 1. Study design.



Prevalence of mastitis pathogens identified both at dry off and at day 3 after calving are shown in Table 1.

Pathogens with the highest prevalence in quarters from high SCC cows at dry off were Staph. aureus, Coalgulase Negative staphylococci, and no growth. Despite farmers being trained in hygienic techniques before the study, contamination of samples was between 19.6-29.2% on average, and varied substantially between farms.

TABLE 1. Prevalence of mastitis causing pathogens at quarter level in % and # of quarters between brackets, in both high and low SCC cows at dry off and after calving.

Treatment Group	At dry off				After Calving			
	H-SCC*		L-SCC*		H-SCC*		L-SCC*	
	N quarters	%	N quarters	%	N quarters	%	N quarters	%
n								
Staph. aureus	40/347	11.5	4/381	1.1	9/315	2.9	4/343	1.2
Strep. uberis	4/347	1.2	0/381	0.0	1/315	0.3	1/343	0.3
Strep. dysgalactiae	0/347	0.0	2/381	0.5	0/315	0.0	1/343	0.3
Lactococcus spp	11/347	3.2	6/381	1.6	2/315	0.6	2/343	0.6
Enterococcus spp	5.347	1.4	7/381	1.8	0/315	0.0	2/343	0.6
E. coli	3/347	0.9	2/381	0.5	4/315	1.3	2/343	0.6
Coagulase negative staphyloc	73/347	21.0	83/381	21.8	29/315	9.2	49/343	14.3
Contaminated	68/347	19.6	86/381	22.6	92/315	29.2	84/343	24.5
No Growth	108/347	31.1	153/381	40.2	155/315	49.2	182/343	53.1

***H-SCC =** 'High' SCC cows (Group A, > 200k cells/ml in at least 1 of the last 3 DHI tests before dry off , n=45), treated with antibiotic + ITS;

L-SCC = 'Low' SCC cows (Group B, < 200k cells/ml in all 3 DHI teste before dry off, n=45), treated with ITS only.

Results of selective dry cow therapy of both treatment groups on cure and prevention of intramammary infections based on bacteriology are shown in Table 2.

This data shows cure rates (85.9% and 91.4%) and prevention rates (75% and 69.4%) as found in this study are in line with results found in the available literature (Swinkels et al., 2021, Rabiee and Lean, 2013).

TABLE 2. Univariate model outcome of quarter level bacteriological cure and prevention rates of the 2 cow level treatment groups, high and low SCC cows at dry off.

	High SCC cows		Low SCC cows	
		95% CI		95% CI
Quarter level cure rate % (# quarters)	85.9 (91/106)	77.7-91.9	91.4 (85/93)	83.8-96.2
Quarter prevention rate % (# quarters)	75.0 (51/68)	63.0-84.7	69.4 (75/108)	59.8-77.9

Authors' affiliation

1. MSD Animal Health, Global Ruminants BU, P.O. Box 31, 5830 AA Boxmeer, The Netherlands.
2. Royal GD, P.O. Box 9, 7400 AA Deventer, the Netherlands.
3. Faculty II, Microbiology, Hannover University of Applied Sciences and Arts, Hannover, Germany.
4. MSD Animal Health, P.O. Box 31, 5830 AA Boxmeer, The Netherlands.
5. Faculty of Health and Medical Sciences, Department of Veterinary and Animal Sciences, Section for Production, Nutrition and Health, University of Copenhagen, Copenhagen, Denmark.

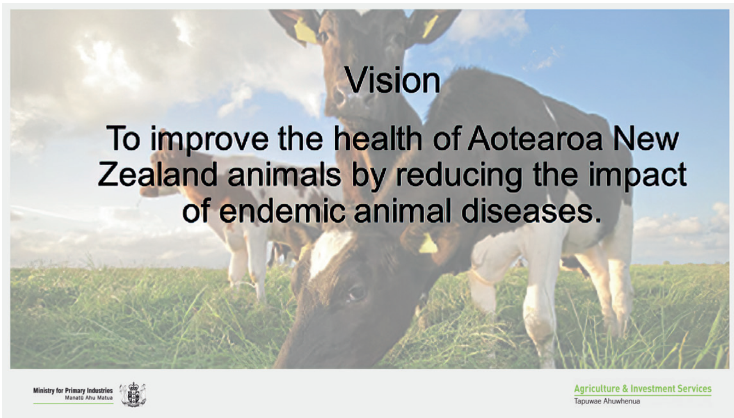
Ministry for Primary Industries Animal Health (Endemic diseases) Team

Kat Govender & Colin O'Connor, MPI

Introduction to the team, its mandate, vision, and scope of work:

The Endemic diseases team was created in March 2021 to fill a gap in MPI's operations which have not included endemic animal diseases other than emerging diseases or those causing human foodborne illnesses, since the late 1990's. The mandate given to the new team was to find areas where MPI could offer support and coordination to existing stakeholder programmes covering endemic animal disease management and control. If the opportunity presented itself the team would fill or bridge gaps.

The teams' vision is to reduce the impact of endemic animal diseases in New Zealand. Outcomes that we want to achieve are increased awareness of endemic animal diseases, improved animal health, better environmental outcomes and increased farm productivity.



The new team of four include a manager and three advisers (one vacant position). The core staff are veterinarians with broad experience in production and companion animals in a clinical and government setting. The team is set to expand in July 2023 to include two regionally based staff.

The scope of work excludes exotic diseases managed under the Biosecurity Act with an internal process to manage emerging diseases. Diseases which are regulated behind the farm gate (bovine tuberculosis and *Mycoplasma bovis*) are also excluded from the scope.

Due to the vast number of endemic diseases and conditions falling within the team's scope and limited resources, the team embarked on a piece of work to objectively determine the diseases of highest impact. The impacts assessed were economic, animal welfare, human health, environmental and socio-cultural. In addition to conducting the objective assessment, the team also consulted widely with stakeholders and ran an on-line survey directed at farmers. The result was a list of 15 diseases and conditions that were ranked according to the impact assessment. These diseases/conditions were further assessed, but not scored, using criteria such as political will, accuracy and availability of tests, ability to manage and status of management, and whether our team could play a role in improving the management status of these diseases/conditions.

The outcome of the impact assessment was that mastitis, bovine salmonellosis and toxoplasmosis are ranked equally with the greatest impact, followed by anthelminthic resistance, lameness and facial eczema ranking jointly second, and bovine viral diarrhoea (BVD) ranking third. However, when assessing BVD together with the other criteria mentioned above, its ranking increased. These results, together with keen interest from industry, stakeholders, veterinarians and results from the farmer survey, influenced the team's decision to propose a multi-year, phased project with the outcome of reducing the national prevalence of BVD to a point where eradication will be possible. All achieved through the voluntary participation of veterinarians and their farmer clients.

BVD Project Design:

The project has been designed to be implemented in three phases, and although some parts of the phases will overlap, the ability to move completely from one phase to the next will depend on achieving specific milestones in each phase.

Prior to obtaining internal approval and seeking funding, the team consulted on the proposal with stakeholders, in particular the BVD steering committee, as a pre-requisite for moving forward. Broad agreement on the objectives of the project and respective roles was achieved, acknowledging that details will need to be worked through as the project progresses through the phases.

Phase 1:

- Governance – a Government (MPI – endemics team) – stakeholder framework
  Milestone: the establishment of a MPI-stakeholder forum
- New on-farm tools
  Milestone: development of a vet/farmer digital platform to input farm data and obtain options for management and a cost/benefit analysis
- Identify and fill gaps in prevalence data
  Milestone: establish a national surveillance programme
- A vet/farmer facing dashboard to show national prevalence (almost real time), and with links to other resources
  Milestone: initiate and develop a dashboard.

Phase 2:

- A data management system and platform (database)
  Milestone: establish a database for herd test results
- Test the capability of the system (management tool, data platform and dashboard)
  Milestone: management tool delivers desired value
  Milestone: herd data can be uploaded seamlessly into a database
  Milestone: the database populates the dashboard continuously
- Confirm test capability for a national programme
  Milestone: test capability is sufficient to meet demand
- Confirm vaccine availability for a national programme
  Milestone: vaccine availability is sufficient to meet demand
- Establish capability for individual animal BVD status recording, including using NAIT
  Milestone: individual and herd status is recorded and available, with controls
- Training
  Milestone: users are proficient in using the tools available.

Phase 3:

- Implementation of National voluntary control programme
- Evaluation of surveillance
- Evaluation of management tools.

Take home message:

Industry, stakeholders, farmers, veterinarians and MPI are all working towards the same goals: to reduce the economic, human health, animal welfare, environmental and socio-cultural impacts of endemic diseases and this is best achieved through a unified and integrated one-health approach. Without regulatory intervention.

Validation of inline SCC for Selective Dry Cow Therapy decision making in dairy cows

Scott McDougall[1,2], Amanda Kilby[3], Jo Holter[3], Rob Orchard[3]

[1] Cognosco, Anexa Animal Health, Morrinsville

[2] School of Veterinary Sciences, Massey University, Palmerston North

[3] MSD Animal Health, Upper Hutt

Introduction

Current New Zealand Veterinary Association guidelines stipulate that antimicrobial dry cow therapy (DCT) should only be used in cows likely to be infected at dry off, while those animals unlikely to be infected should be infused with an internal teat sealant (i.e. "selective" DCT). As culture of every cow is not economically or logistically feasible, clinical mastitis treatment records and herd test somatic cell count (SCC) data have been used to select cows likely infected (i.e. those with a history of clinical mastitis and/or a maximum herd test SCC >150,000 cells/mL for cows, and >120,000 cells/mL for heifers) as selection criteria. However, about a quarter of herds nationally do not herd test. Additionally, the frequency and timing of herd tests may be such that concerns may be raised about the sensitivity (Se) and specificity (Sp) of herd test data in defining intramammary infection (IMI) at drying off. Automated inline SCC testing using principles similar to that of the rapid mastitis test is available (Allflex SCC, formerly known as CellSense). In New Zealand, these devices are generally placed at a quarter of the bails in a dairy shed and hence provide SCC data at every milking for a quarter of the cows in the population. The objective of this study was to assess the test characteristics of Allflex SCC data in predicting presence of any IMI, or an IMI associated with a major pathogen (i.e. *Staphylococcus aureus, Streptococcus dysgalactiae,* or *Streptococcus uberis*) at the end of lactation.

Materials and methods

Cows (n = 1,544) from four seasonal calving dairy herds (two in the Waikato, one in Taranaki, one in Southland) were enrolled in the study on the basis of having Allflex SCC systems and willingness to undertake monthly herd testing throughout lactation. On the day of drying off cow-composite milk samples were created by pooling ~1 mL of milk from each quarter into a single vial following aseptic preparation of the teat end followed by discarding the first three squirts of milk. Cows were defined as uninfected, infected with a minor pathogen or infected with a major pathogen based on bacteriology.

Receiver operator curves (ROC), that is the sensitivity (Se) and 1- specificity (Sp), were plotted for each potential cutpoint using the last and maximum herd test SCC and the bounded geometric mean for all data points in the last 12 weeks of lactation for the Allflex SCC data, and the area under the curve (AUC) was calculated. Differences in the AUC were compared statistically testing for superiority (roccomp in STATA), and non-inferiority (rocNIT in R).

Results

The AUC of the ROC was greater (p=0.03) for the geometric mean 12-week Allflex SCC than the last or maximum herd test SCC for any IMI (Figure 1a, Table 1). The AUC did not differ between the different diagnostic tests for any IMI associated with a major pathogen infection (Figure 1b, Table 1). The AUC for the 12 week Allflex SCC was non-inferior to that for the maximum or last herd test SCC for any IMI, and for major pathogen IMI (p<0.05 for all comparisons).

Figure 1. Receiver operator curve for presence of (a) any intramammary infection, or (b) a major pathogen intramammary infection at drying off using either the geometric mean of the Allflex SCC over the last 12 weeks of lactation (red triangle), the SCC at the last herd test (blue circle) or the maximum SCC across lactation (yellow square).

Figure 1.

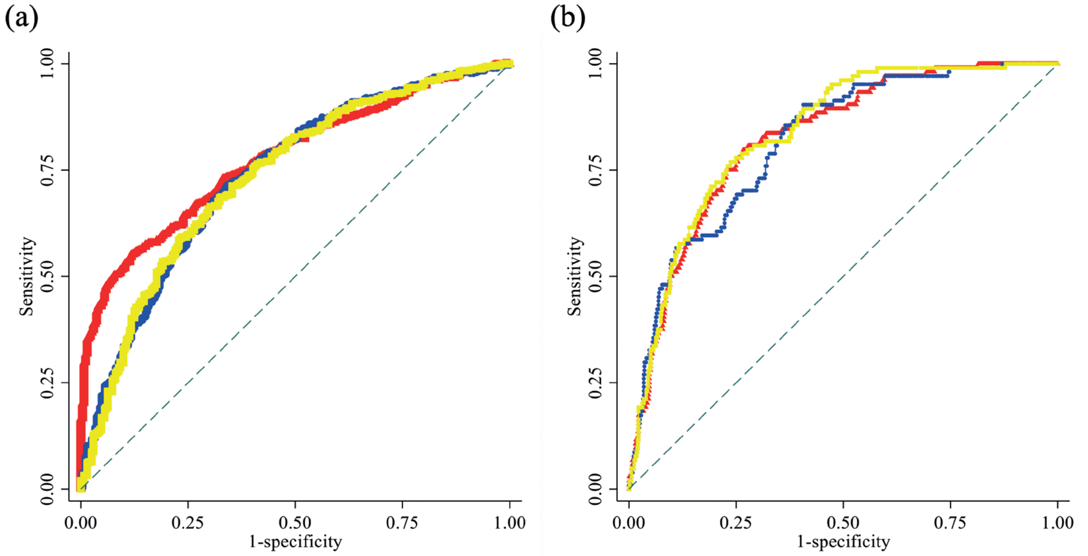


Table 1. The estimated area under the curve (AUC), the standard error of the estimate of the area under the curve (SE) and the 95% confidence intervals for the AUC where the geometric mean of Allflex SCC during the last 12 weeks of lactation, the SCC at the last herd test of lactation (Last HT SCC) or the maximum lactational herd test SCC (Max HT SCC) were used to predict the presence of any intramammary infection or of a major intramammary infection at drying off. The p-values are for the superiority comparison of the area under the curve for the Allflex SCC estimate compared with the last SCC and compared with the maximum SCC are presented.

Table 1.

				95% CI		Allflex SCC vs	Allflex SCC vs
		AUC	SE	low	high	last SCC	max SCC
Any IMI	Allflex SCC	0.78	0.02	0.74	0.81	0.03	0.03
	Last HT SCC	0.74	0.02	0.70	0.77		
	Max HT SCC	0.74	0.02	0.70	0.77		
Major IMI	Allflex SCC	0.82	0.02	0.79	0.86	ns	ns
	Last HT SCC	0.82	0.02	0.78	0.86		
	Max HT SCC	0.84	0.02	0.80	0.87		

Conclusions

It is concluded that use of the geometric mean Allflex SCC data from the last 12 weeks of lactation results in a superior AUC than either the maximum or last herd test SCC for detection of any IMI at drying off. The three different tests did not differ in AUC in detecting major pathogen IMI. The Allflex SCC was noninferior to the maximum and last herd test SCC.

Thus, use of Allflex SCC data to select cows for antimicrobial DCT or internal teat sealant at the time of drying off will result in equivalent or superior ability to differentiate infected from non-infected cows compared with use of herd test SCC data.

Mastitis Management using Milk Sensors

Rob Orchard, MSD Animal Health Intelligence

Automated electronic sensors are increasingly used to monitor the health and performance of farm animals, and on-line milk sensing is an important part of this trend. Allflex offers two milk sensors as part of its Protrack® product suite, each analysing the milk of individual cows in real time. The 'Milk' sensor collects volume, fat, protein, lactose, conductivity, blood in milk and watery milk data, while the 'SCC' sensor estimates the somatic cell count (SCC) using an automated rapid mastitis test. Both products have been on the market for more than 15 years.

Figure 1: Allflex 'Milk' (left) and 'SCC' (right) sensors

Protrack in-shed software includes some basic features enabling the farmer to make management decisions from milk data. Alerts can be automatically generated so that cows can be inspected on their next visit to the shed. Reports are available that allow the farmer to rank cows according to averaged sensor results, and to drill down to view a cow's data history (e.g.,Figure 2). These basic features are a good start, but there is a desire to bring sensor data reporting to the next level – to create a series of decision-oriented tools that streamline decision making for both farmers and vets.

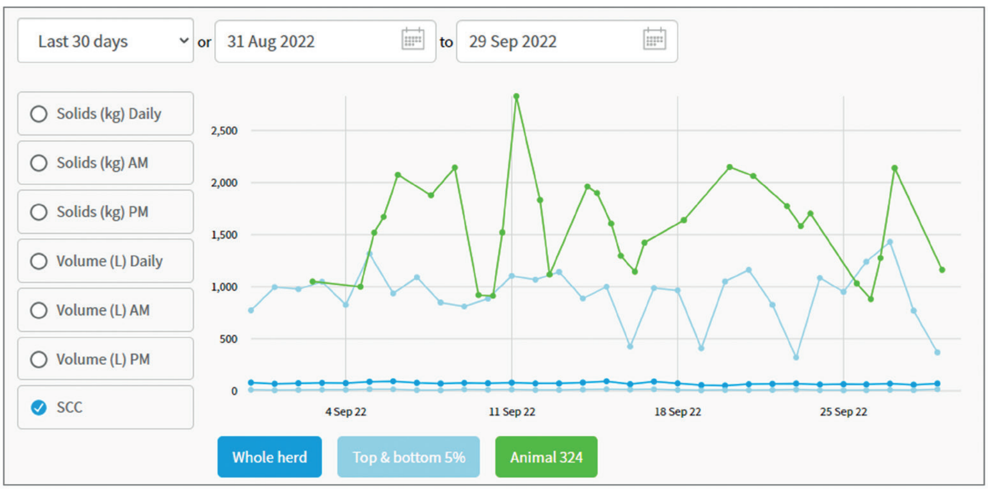


Figure 2: Protrack screen showing the last 30 days SCC data for cow 324.

The availability of frequent milk-quality data has the potential to change how mastitis can be managed on farm. The purpose of this session is to present some concepts for how we could best use frequent sensor data for mastitis management and get feedback from veterinarians about what they would like to see.

Individual cow mastitis management: What information about an individual cow would you like when considering a mastitis treatment or other action? Would you manage an individual case of subclinical mastitis differently if you knew it was a recent infection?

Herd mastitis management: What herd statistics would you like when evaluating the overall mastitis management of a herd?

Managing bulk tank SCC: We could identify the highest SCC contributors to the bulk tank and model the impact of removing specific cows on the bulk tank SCC and predict the reduction in milk solids.

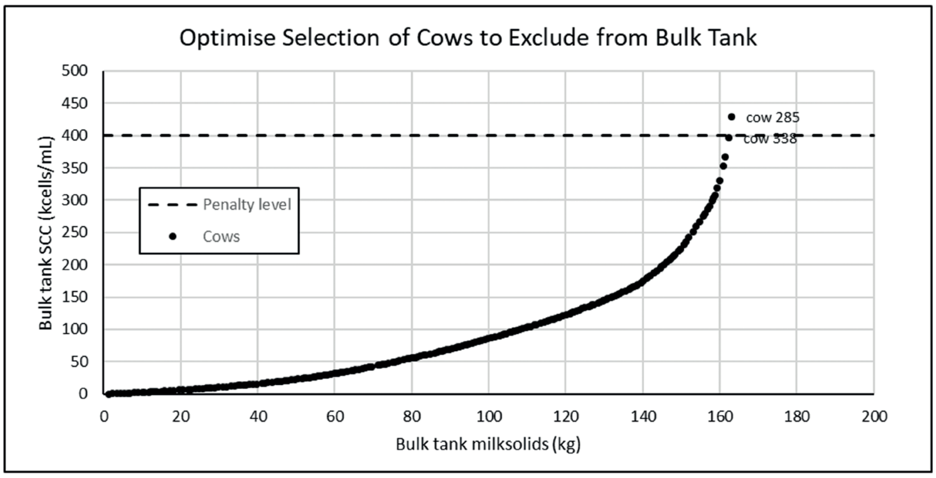


Figure 3: Concept cow-selection tool for managing bulk tank SCC. The plot shows the cumulative bulk tank totals after each cow is added. Removing the cows at the top right is the most efficient way to quickly reduce bulk tank SCC.

Detecting clinical mastitis: We could provide cow-lists and in-shed alerts for cows most at risk, based on very high SCC or other mastitis alerts (blood, conductivity, watery milk, yield dip).

Selecting cows for dry-off: Mastitis is a factor in the selection of cows for dry-off, with contribution to bulk tank SCC a key consideration. What other mastitis-related considerations should be part this decision?

Selective dry-cow therapy: We could identify cows likely to have an intra-mammary infection, so they can receive dry-cow therapy.

In summary, there are a number of cow and herd management decisions related to mastitis, each of which may require subtly different presentation of data to enable optimum decision making. How would your approach to mastitis management change given the availability of frequent milk-quality data? Focusing on each decision-type, how would you use frequent milk-quality data to make the best decisions?

Tips for Transition Success

Ryan Luckman, Oamaru Vets

The transition period offers a key opportunity to engage with collar data, in a period that has meaningful impact on cow health, milk production, and reproductive success. Using the Transition and Rumination Dashboards from the MSD Animal Health Intelligence Elite Package we've been able to set our farmers up with target recovery lines, and then monitor their performance and trouble shoot if they fall short. Some of the common issues have been:

Springers

In our Collar Repro Reviews this season, one of the major findings was that underfeeding of Springers had a massive impact on early season health, as well as pre-mate cycling rates. We know that during the calving process cows stop eating, so subsequently their rumination levels fall to the lowest point on the day of calving (day 0), with drops of up to 50% typically seen.

With our typical diets in South Canterbury it appears that farms should be aiming for a minimum rumination target of around 450 minutes per day in the Springers. Farms that reached this level were often able to reach average Day 0 rumination rates of ~330 minutes/day.

When looking at the Springers we need to also consider the energy component of the diet. Rumination rates increase with high fibre diets, so you may be able to reach target rates with 6kg of straw, but the reserve energy will still be limiting. We therefore recommend first sitting down and working out the energy in the diet, with a target of feeding 90-100% of maintenance energy (down the throat) as per the DairyNZ recommendations. We found a lot of farms feeding just 60% of maintenance this season, with the "keep springers tight" mentality being taken to the extreme.

For monitoring we will sit down with our clients and first use an energy calculator to check that the proposed diet will reach the 90% minimum target. We've seen that typically a diet needs to have around 10kg of green fed component (silage/ grass / maize etc), plus 1-2kg of straw to hit target. Daily monitoring via the Rumination Dashboard (Springers Report) then allows us to check if the proposed diet is actually being fed.

Transition

Post-calving rumination rates are a function of both feed offered AND voluntary feed intake. On our farms we are targeting getting herds up to rumination targets of 400-450 minutes per day as soon as possible, with minimum recovery levels of 380 minutes per day.

The use of OAD milking in the post-calving period has improved the recovery lines on a lot of farms, as well as decreasing endometritis rates, early lactation health alerts, and improving reproductive performance. However not all OAD farms had good outcomes – when we investigated these farms we found six common issues across our farms:

1. **Underfeeding of Springer Cows** – these cows had low rumination rates on the day of calving and took longer to bounce back
2. **Leaving freshly calved cows on the yard** (rather than drop in paddock)
3. **Under-allocation of feed** to the colostrum mob as more cows were added
4. **Grazing to residuals lower than 1800kgDM**
5. **Use of 24 hour grazing management** – the most successful farms had 3 or more feed offerings (i.e 2x grass breaks, 1x silage)
6. **Insufficient limeflour** – successful farms were using 250g limeflour in the colostrum mob

Using the Transition Report on the Transition Dashboard you can identify if farms are reaching target, and if not identify any specific days a farm is struggling (i.e day 5 when transferring between mobs). The report is very responsive, so you get feedback the following day as to whether the changes you've made have been successful.

Overall transition offers an easy entry point for working with collar data, at a period where changes have a huge impact on the outcome of the season. Farmers are often great at identifying what changes they can make to improve things, so the key role of the vet is to identify the timing of issues, and offer a sounding board of what other farms are doing that's working.

Cow Monitoring Collars 101

Amanda Kilby, (VMD, MA) Allflex / MSD Animal Health Intelligence Technical Veterinarian

Monitoring technology is rapidly being adopted by dairy farmers in New Zealand and is therefore capturing the attention of most dairy veterinarians. Various applications of the technology were referenced in the dairy stream of the NZVA conference in June[1] and it seems timely to follow up with an overview of the technology.

The Basics

On Allflex collars, the blue 'tag' on the left side of the collar strap is where the technology lies. Inside the tag is an accelerometer, which tracks rumination and other behavioural states. The raw data is sent every 20 minutes to 'readers,' which are hardware receivers, positioned on the dairy shed or on mobile reading stations, such as trailers with solar panels.

By comparing cows to their own individual behavioral baseline and some general expectations of rumination, the software identifies behavioral abnormalities, and uses these to categorize cows as on heat, in ill health, and in distress.

For the system to work as expected, the farmer must ensure daily (manual) data entry of calvings and matings, and reasonably timely entry of pregnancy test results, dry-off, and culling. These lifecycle events, along with all the system-generated heat events, health events, and distress events, as well as anything else the farmer chooses to input (eg. group movements, animal health treatments etc.), are stored for the life of the cow and for three years after they are culled.

All this information can be viewed on various reports (lists of cows that meet specific criteria), portals (dashboards of related reports) and/or via alerts (flashing light on the software and/or push notifications to the farmer's phone). The database can be queried and reports built to suit each farm. This allows each farm to customize their system and allows farm stakeholders, such as veterinarians, to perform retrospective data analysis or provide customized consulting in real time.

What's normal?

One of the challenges when first presented with monitoring data is to determine what is normal. How long does a healthy cow ruminate in a 24-hour period? How does calving, weather and health events impact rumination?

Average sized grazing dairy cows ruminate 450-550 minutes per day[2]. Rumination minutes are driven by medium particle size non-digestible fibre (NDF) intake, so need to be interpreted in light of current diet.

It is 'normal' for a cow's rumination to drop by 30-50% on the day of calving[3] but it should then recover quickly (within 4-7 days) to near the cow's pre-calving baseline.

Ideally, rumination should be consistent from day to day at both cow level and mob/herd level. For example, more than 50 minutes of mob-level rumination variation from day-to-day indicates variable feed quantity or quality and could impact feed efficiency.

What are the applications?

Overseas, studies have shown that this technology can correctly identify 90% of all heats[4]. It has also been used to detect health events, such as displaced abomasums, ketosis and metritis 1-3 days before an experienced farm worker would[5,6,7].

Together, these system functions help scale the skilled labor on the farm and provide peace of mind by acting like another set of eyes watching the cows.

For farms that struggle with heat detection, the collars can help make the move away from bulls, shorten the mating period, improve submission rates and 3-week-in-calf rates, and can pick up weak heats and returns late in the mating season, when human fatigue usually kicks in.

For farms that already do well with heat detection, the collars ensure consistently excellent heat detection without hours of daily human input.

What’s next?

While most farmers buy collars initially for heat detection, many soon want to engage more with the rumination and health data. But, making decisions based on rumination and health alerts is a bit more nuanced than using the system solely for heat detection, so it is important that the farmer work with their veterinarian and/or nutritionist to interpret the data.

To help mediate this process, Allflex / MSD Animal Health Intelligence has commercialised a set of portals. If the farmer purchases them, these seasonal portals (aka dashboards of reports) are added to the farmer’s existing software interface. They are designed to both automate daily decision making on farm (for example: “has this cow recovered rumination sufficiently to go from a once-a-day mob to a twice-a-day mob after calving?”) and to help with retrospective analysis and/or meta-analysis of data from multiple farms by a veterinarian or nutritionist.

But a farm doesn’t have to purchase the portals to get their veterinarian involved with monitoring. Other opportunities for veterinarians to engage with collared herds include:

- Developing treatment protocols for cows on the Health and Distress reports
- Farm worker training on basics of sick cow exams, cow comfort assessments, rumination/nutrition 101
- Using the reports to find cows eligible for reproductive interventions or identify problems with non-cyclers or phantom cows early
- Monitor patterns to pick up herd level problems, like subclinical hypocalcemia or ketosis, sooner
- Use collar data alongside Infovet, MINDA LIVE and/or milk supplier information to enhance repro reviews, animal welfare assessments and milk quality consults

While it takes a bit of time to get your head around the technology, once you do, you can treat every cow as an individual, gather unbiased data consistently, and get closer to your clients by offering them more preventative service and advice.

If you need further information after reading this article, feel free to contact me directly to arrange some training.

References:

1. Proceedings of the Dairy Veterinarians of the NZVA, 2022 conference: https://www.sciquest.org.nz/browse/publications/view/87
2. Pudyal, S. (2021). Using rumination time to manage health and reproduction in dairy cattle: a review. Veterinary Quarterly. 41:1. 292–300. https://doi.org/10.1080/01652176.2021.1987581
3. Pahl, C *et al.* (2014). Rumination activity of dairy cows in the 24 hours before and after calving. J. Dairy Sci. 97 :6935–6941. http://dx.doi.org/10.3168/jds.2014-8194
4. Madureira, AML et al. (2015). Factors affecting expression of estrus measured by activity monitors and conception risk of lactating dairy cows. J. Dairy Sci. 98:7003–7014. http://dx.doi.org/10.3168/jds.2015-9672
5. Stangaferro, ML et al. (2016). Use of rumination and activity monitoring for the identification of dairy cows with health disorders: Part I. Metabolic and digestive disorders. J. Dairy Sci. 99:7395–7410. http://dx.doi.org/10.3168/jds.2016-10907
6. Stangaferro, ML *et al.* (2016). Use of rumination and activity monitoring for the identification of dairy cows with health disorders: Part III. Metritis. J. Dairy Sci. 99:7422–7433 http://dx.doi.org/10.3168/jds.2016-11352
7. Kaufman, EI *et al.* (2016). Association of rumination time with subclinical ketosis in transition dairy cows. J. Dairy Sci. 99:5604–5618. http://dx.doi.org/10.3168/jds.2015-10509

Notes:

Notes:

Looking after kiwi farms for generations.

We're excited to introduce a new sustainability initiative in collaboration with Agrecovery aiming to help reduce plastic waste on New Zealand farms.

Let's continue looking after our farms so our children and grandchildren can do the same.

You can drop empty MSD Vaccine Vaxipacks® in collection bins at participating vet clinics and rural retailers. We'll take care of the rest, ensuring the plastic is repurposed and doesn't end up in landfill.

To find out more talk to your local vet clinic or rural retailer or visit: www.sheepvax.co.nz/recycling

Recycle MSD Vaxipacks® from the following products:

MULTINE® B12	MULTINE®	NILVAX®	LAMB VACCINE
COVEXIN 10	YERSINIAVAX®	TOXOVAX®	CAMPYVAX4®
SALVEXIN®+B	ANDROVAX® plus	FOOTVAX®	LEPTAVOID® 2