MEAT & LIVESTOCK AUSTRALIA

final report

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Feedlot Animal Health Management Program

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Executive summary

The Australian feedlot industry is committed to retaining its status as a supplier of safe and nutritious beef into the future. Maximising animal health and welfare outcomes, preservation of key domestic and international markets and the maintenance of human health are key imperatives for the Australian feedlot industry. Accordingly, the Australian feedlot industry has developed a comprehensive Animal Health Management Program.

This program will ensure feedlots have access to information on evidence-based infection prevention and control measures, and ensure that when animal health treatments are required, that they are used appropriately and prudently to minimise the potential development of antimicrobial resistance (AMR) in both cattle and humans. With the increasing importance of antimicrobial stewardship, a multidisciplinary team was assembled comprising antimicrobial stewardship experts, feedlot veterinary clinicians and university veterinary microbiologists to develop the program. The following elements have been produced for the Australian feedlot industry:

1. A situation review on antimicrobial resistance for the feedlot industry.
2. Survey of the Australian feedlot industry on antimicrobial use and practices.
3. Development of antimicrobial stewardship guidelines and framework for the Australian Cattle Feedlot Industry.
4. Literature review of alternatives to antibiotics for the prevention and treatment of commonly occurring feedlot diseases.
5. Development of eLearning courses for antimicrobial stewardship for the feedlot industry
6. Development of an outline and training materials on antimicrobial stewardship for the feedlot industry.

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1 Background

MLA and the Australian Feedlot Industry recognise the need for resource materials for feedlot operators, management and staff, as well as veterinarians servicing the industry, to assist in maximising animal health and welfare outcomes that are synonymous with retaining its status as a supplier of safe and nutritious beef now and into the future. The value proposition for the Australian feedlot industry is that through achieving these outcomes, there is also maintenance of maximum human health and preservation of key domestic and international markets. MLA and the Australian Feedlot Industry are conscious of the fact that these resource materials should include a spectrum of animal health management principles, such as evolving diagnostic technologies, husbandry considerations and nutritional aspects, and not just pharmaceutical treatments. Infection prevention and control measures utilised in food producing animals are prominent in the public consciousness and are key considerations for feedlot operators and veterinary practitioners to achieve maximum animal health and welfare outcomes. Importantly, the Australian Government "National Antimicrobial Resistance Strategy 2015-2019" recognises the importance of infection prevention and control measures which are one of the key objectives of the strategy. MLA and the Australian Feedlot Industry also recognise the need for resource materials to be available for feedlots and relevant stakeholders to the industry which provide evidence-based information for the appropriate and prudent use of antimicrobials that minimise the potential development of antimicrobial resistance (AMR) in both cattle and humans.

2 Project objectives

The aim of this project was the production of a comprehensive Animal Health Management Program, comprising written and illustrated reference material, as well as audio-visual and other training resources on all aspects of animal health management in the feedlot environment. Different components of the program will be suitable for use by feedlot veterinarians, feedlot operators, feedlot managers and feedlot staff. The materials will take into account Australia's animal welfare standards and guidelines for cattle, requirements of the National Feedlot Accreditation Scheme, the National Antimicrobial Resistance Strategy (2015-2019), legal provisions of the relevant jurisdictions and also the Australian Veterinary Association Guidelines for Prescribing, Authorising and Dispensing Veterinary Medicines (2005). Specific objectives included production of the following materials:

2.1 Technical Manual

Produce an illustrated and referenced, technical manual containing comprehensive information on Animal Health Management in Australian feedlots. The detailed reference manual will cover all aspects of the legislative framework in the various jurisdictions, pharmaceutical and non-pharmaceutical principles of animal health management, husbandry practices and infrastructure considerations, preventative programs, current and emerging diagnostic modalities, alternative therapies and quality assurance programs relevant to the feedlot industry that aim to minimise risk of disease entry and spread. There will also be detailed focus on current best practice on feedlots to ensure the appropriate and judicious prescription, dispensing and administering of animal health treatments – including antimicrobials – and mechanisms for on-feedlot and post-feedlot surveillance of antimicrobial susceptibility. An antimicrobial stewardship program for the Australian feedlot industry will be designed and recommended. The best practice recommendations delivered in the manual will cater for future iterations and improvements, based on the evolving science, with the aim always towards ensuring the industry's responsible use of animal health treatments and maximising treatment success. Residue testing programs available for antimicrobials and other relevant animal health treatments available domestically and abroad will be discussed. The technical

manual will also address the need for industry standards of terminology, collection of information, measurements, observations, recording, accessing information, and information quality and verification requirements as they relate to the subject matter. Complimentary industry programs such as NFAS, LPA, MSA, NLIS and NRS will also be discussed.

2.2 Situation Review

Produce a situation review containing comprehensive information on (i) Classes of animal health treatments (both antimicrobials and ionophores) utilised by the feedlot industry for treatment of infectious disease and metaphylaxis management (both in-feed and injectable); (ii) Risk of antimicrobials utilised by the feedlot industry to cause AMR in human medicine; and (iii) Documentation of research conducted on antimicrobial resistance in the Australian feedlot industry, and any links to AMR in human or veterinary medicine together with comparisons of this work with research conducted on AMR with any other livestock sectors and in feedlots internationally.

2.3 Industry Training Modules

Produce Industry Training Modules that are designed to complement existing industry training programs and material and formatted to include:

1. Trainer notes and references
2. Participant workbooks
3. Summary fact sheets
4. Example “work instructions”
5. Interactive online resources (including short webinars / videos)
6. Quiz and assessment material

2.4 Training Course

Produce a detailed outline of a 1-day training course (including course content, timetable, class layout, props and draft certificate) where the products of this project may form part of the training materials.

2.5 Industry Extension Material

Produce Industry Extension Material and Delivery and Communication Program that defines and documents a detailed plan for getting the project material out to the feedlot industry and the process for measurement of the uptake by the feedlot industry. Part of the measurement process would be a registry of organisations and individuals that have completed training modules and their respective assessment results.

3 Methodology

3.1 Project Consultants

The project was undertaken by five veterinarians with relevant experience as practitioners in the feedlot industry [KS, PC & DF] or with advanced veterinary qualifications in pharmacology [SP] and

microbiology [DT] who work in research and tertiary veterinary education, and are recognised experts and publishers in the field of antimicrobial resistance, antimicrobial stewardship and antimicrobial prudent use.

3.2 Project Management

An inception meeting with MLA and steering committee was undertaken to confirm project scope and deliverables.

3.3 External Review

An external reviewer (a recognised veterinary expert in the field of bovine medicine and therapeutics with international experience) was engaged at the draft stage of development of the technical manual and the situation review document.

3.4 Situation Review

All research conducted on antimicrobial resistance in the Australian feedlot industry, and any links to AMR in human or veterinary medicine, was researched and analysed following a literature search and consultation with microbiology and industry experts. Comparison of this work with research conducted on AMR with other livestock sectors and in feedlots internationally was undertaken. Antimicrobial agents approved by the APVMA for use in feedlot cattle were extracted from the regulator's database and tabulated together with information on ASTAG importance rating. Similarly, a list of all vaccines approved for use in feedlot cattle was prepared based on the content of the APVMA product database.

3.5 Technical Manual

The technical reference manual and the situation review were delivered first in draft form to MLA and the steering committee and upon acceptance were finalised. All other deliverables were derived from these two documents.

The manual was prepared following engagement of other feedlot veterinarians outside the project team and obtaining their feedback combined with the project teams' knowledge base, building consensus to document current best practice arrangements on Australian feedlots to ensure the appropriate and judicious diagnosis, prescription, dispensing and administering of animal health treatments including antimicrobials. The current framework for storage and access to animal health treatments by feedlot staff was determined. A literature review was undertaken to incorporate a wider and more global perspective including a review of the legislative framework of individual animal health treatment classes per the project objectives

Technologies currently utilised in the feedlot industry for timely and accurate diagnosis of sick animals to ensure the judicious use of animal health treatments (for example pen riding and visual observations, rectal thermometers, "Whisper" electronic stethoscope, advanced software systems to detect disease onset) were documented and the diagnostic procedures list was augmented and a literature review undertaken to incorporate a wider and more global perspective.

Alternative animal health treatments available for the treatment or prevention of disease in feedlot cattle was investigated by consultation with the broader group of feedlot personell combined with a literature review that was undertaken to identify, retrieve and analyse published information to provide a wider and more global perspective.

Current best practice infrastructure and programs present in the feedlot industry to decrease animal stress, maintain or improve the health of cattle, hence minimising the need for treatment interventions (for example backgrounding programs, vaccination, handling facilities, hospital facilities, stock handling and biosecurity practices) were reviewed after consultation with the feedlot veterinarians. The information from the consultation was extened with a literature review to incorporate a wider and more global perspective.

Feedlot industry quality assurance program requirements to minimise the risk of disease entry and spread and ensure the responsible use of animal health treatments (for example NFAS) were reviewed and documented. Other livestock industry quality assurance programs such as those that exist for the pork and dairy industry, the live export industry and beef feedlot industry quality assurance programs existing abroad were also be reviewed to give a wider perspective

Residue testing programs for antimicrobials (for example the Australian National Residue Survey and residue testing programs in importing countries such as that of the USDA FSIS) were reviewed and documented.

3.6 Review Of Antimicrobial Use

3.6.1 Study population

The target population for the survey was beef feedlots operating in Australia in 2017. A composite list of 517 feedlots was generated from several sources, primarily consisting of the client databases of five veterinary consultancy practices based on the east coast of Australia (the source population). The eligible population comprised those beef feedlot operators who consented to participate in the study. Membership of the National Feedlot Accreditation Scheme, a voluntary quality assurance scheme for the Australian lotfeeding industry, was not a prerequisite for participation in the survey. Operators were encouraged to participate via an introductory information pack provided by the peak industry body, the Australian Lot Feeders Association, and Meat & Livestock Australia. The information pack included an introductory letter from Meat & Livestock Australia notifying the feedlot of the impending survey, and fact sheets on antimicrobial use and stewardship in the cattle industry. Each feedlot was also given a unique code at the time of the mail-out which enabled them to respond anonymously to the questionnaire, with the research team being blinded to the identity of each feedlot during analysis and interpretation.

3.6.2 Questionnaire

The questionnaire was developed by a group of four experienced beef feedlot veterinarians and two veterinarians with expertise in antimicrobial resistance ecology and antimicrobial stewardship (see supplementary materials). The questionnaire was created online using Qualtrics survey software (qualtrics.com/au/) and consisted of 98 questions grouped into five sections:

Section 1: Feedlot general information. This section asked for background information including the size of the feedlot, the number of animals sold, the average days-on-feed, and the percentage of total animals that required treatment in a hospital pen in the previous 12-month period (February 2016-February 2017).

Section 2: Antimicrobial use. Respondents answered a series of questions related to antimicrobial use in the previous 12 months, specifically whether certain antimicrobials had been used. A total of 26 antimicrobials (injectable and in-feed) were listed. The antimicrobials included all antibiotic classes and comprised a cross-section of drugs considered to be of low, medium, and high importance to human and veterinary medicine. If an antimicrobial was used, the respondent was asked to estimate the percentage of animals treated and to nominate the purpose of use (individual treatment, mass treatment (i.e. in response to a disease outbreak (metaphylaxis)), timed treatment (i.e. the timed/scheduled treatment of lots (prophylaxis)), prevention, or growth promotion) and disease syndrome/s treated (respiratory, digestive, musculoskeletal, neurological, urogenital, 'other diseases').

Section 3: Veterinary treatment protocols. Included questions on frequency of veterinary visits, the presence of protocols for (i) newly introduced animals, and (ii) veterinary medicines/ treatment, whether protocols were followed by feedlot staff, and if sick animals were assessed for response to treatments.

Section 4: Supply and use of veterinary chemicals. Respondents were required to answer a series of questions related to the supply and purchase of prescription and over-the-counter animal health products, access and administration of veterinary chemicals, identification of treated animals and training in the administration of veterinary chemicals.
Section 5: Storage and chemical stock control. This section included questions on the storage and auditing of veterinary chemicals.

All questions were closed-ended, multiple-choice questions, except for questions where 'other' could be selected, and a respondent could elaborate if desired. For all questions, the response options of "don't know" and "unanswered" were available.

The questionnaire was pilot-tested among five feedlot operators using telephone interviews. These interviews took on average 20 to 25 minutes to complete. Following feedback from pilot-testing, minor amendments to the questionnaire were made and a paper-based version (comprised of 14 pages) sent by post was adopted to allow respondents more time to complete the questionnaire and consult herd records where necessary. The paper version of the final questionnaire was mailed to 517 beef feedlot operators in February 2017. Feedlots who did not respond within three to four weeks after being sent the questionnaire by post were emailed an electronic version. The collection period for responses was 13 February 2017 to 1 July 2017. Data from returned questionnaires was entered manually into the Qualtrics online form by trained staff members of one of the authors. If required, an intermediary not associated with the data analysis, contacted a respondent to clarify any responses that were difficult to interpret.

3.6.3 Statistical analysis

Data were downloaded from the survey software platform as a comma separated values file into MS Excel and then imported into Stata version 15.0 (Stata Corporation, College Station, TX) for analysis. Descriptive statistics were calculated using unadjusted frequency counts, with proportions reported as the number of respondents selecting an answer-option divided by the total number of respondents attempting the question. Responses of "don't know" and "unanswered" were

interpreted as missing values (not included in the analysis). Some response categories were merged when redundant or to simplify interpretation. Odds ratios were computed from contingency tables and the Fishers' exact test was performed with associated two-sided P-values reported as significant at less than the five percent level.

3.7 Training Modules

Industry training modules for the Animal Health Management Program involved team planning, assembly of reference materials, drafting, producing graphics and scripts before filming of a series of short webinars.

4 Results

The results and outcomes of the project are set out in the 10 appendices to this report.

5 Discussion

This project reinforces the feedlot sector's position as being an industry which currently has low risk of AMR and provides a mechanism for the maintenance of a low AMR risk in formats highly visible to the consumers of feedlot beef. In addition, the potential rewards of this project include better welfare and treatment outcomes for feedlot cattle, increased competence and upskilling of feedlot staff, better industry knowledge and awareness of this complex topic and protection of the industry's reputation. Further potential rewards include better feedlot level diagnosis, case definition and industry standards in animal health terminology, measurement, and data collection. The project outcomes deliver a means of reducing overall antimicrobial usage and fostering the current best practice usage of antimicrobial agents. These combined benefits provide significant direct and indirect ongoing commercial benefit to the feedlot industry.

5.1 Situation Review

A review of the current status of the use of antimicrobials and the level of antimicrobial resistance in the feedlot industry was undertaken. The review considered foodborne pathogens, commensal bacteria and bovine respiratory disease bacteria. The review found that there have been very few studies in AMR conducted in the Australian feedlot sector. Those that have been undertaken have confirmed very low levels of AMR in foodborne pathogens and commensals in healthy feedlot animals at slaughter. Importantly, no resistance to critically important antimicrobials was found. Resistance to third generation cephalosporins has recently been confirmed among multidrug resistant Salmonella isolates from dairy cattle and dairy calves in Australia. There are no recent Australian studies on the antimicrobial susceptibility profiles of *Mannheimia haemolytica* and *Pasteurella multocida* in feedlot cattle. A recent study of *Histophilus somni* isolates from Australia found only a single isolate showing resistance to tetracycline. The full text of the situation review can be found in **Appendix 1.**

5.2 Feedlot Survey

A survey of Australian feedlots was conducted to ascertain the antimicrobial usage in Australian feedlots. 517 feedlots were contacted to see if they would consent to participate in the survey. Feedlot operators were encouraged to participate via an introductory information pack provided by

the Australian Lot Feeders Association (ALFA) and Meat and Livestock Australia (MLA). The information pack included an introductory letter from Meat and Livestock Australia notifying the feedlot of the impending survey and a fact sheet on antimicrobial use and stewardship in the cattle industry. Each feedlot was given a unique code at the time of the mailout which enabled them to respond anonymously to the questionnaire with the research team being blinded to the identity of each feedlot during analysis and interpretation. The questionnaire was developed by a group of four experienced beef feedlot veterinarians and two veterinarians with expertise in antimicrobial resistance ecology and antimicrobial stewardship. The survey contained 98 questions grouped into 5 sections.

Section 1. Feedlot general information
Section 2. Antimicrobial use
Section 3. Veterinary treatment protocols
Section 4. Supply and use of veterinary chemicals
Section 5. Storage and veterinary chemical stock control.

The questionnaire is contained in **Appendix 2.**

5.3 Antimicrobial use in the Australian beef feedlot industry: results from a national pilot survey.

This is the first survey of antimicrobial use in Australian beef feedlots. The operation-level descriptive estimates of antimicrobial usage described here are a starting point for further research aimed at generating accurate quantitative estimates of antimicrobial use at the animal-level, and for identifying veterinary and owner motivations for antimicrobial use. Ideally, surveillance of antimicrobial usage would occur alongside surveillance of antimicrobial resistance, thereby enabling the design and implementation of optimal strategies to control antimicrobial resistance in the beef feedlot sector. The full report on the results of the Antimicrobial use in the Australian Beef Feedlot Industry Survey is currently submitted for a peer reviewed review journal publication (Aust Vet J 2019 doi: 10.1111/avj.12889).

5.4 Antimicrobial Alternatives

Replacement of antimicrobials should be considered whenever available evidence supports the efficacy, safety and low or absent potential to select for antimicrobial resistance of the alternative. There has been substantial interest for more than two decades to find alternatives to antibiotics for use in cattle and other livestock species. Prebiotics, probiotics, synbiotics, eubiotics, competitive exclusion, antibodies, immunomodulators, bacteriophages, predatory bacteria, phytochemicals (chemicals obtained from plants), antimicrobial peptides, clays (including zeolites), minerals and other approaches have been investigated. A recent review by the authors on alternatives to antibiotics in feedlot cattle showed further research is warranted into bacteriophages, nitric oxide, supplemental yeast and yeast products in high fibre starter diets, and direct fed bacteria. Whilst none of these alternatives to antibiotics have robust supporting data to date, an important task of the antimicrobial stewardship team is to identify those alternatives with sufficient evidence to be considered as replacements for antimicrobials as evidence accrues over time. The literature review conducted on the alternatives to antimicrobials in feedlot cattle is contained in **Appendix 3.**

5.5 Antimicrobial Stewardship Framework

Antimicrobial use and antimicrobial resistance in both humans and animals are high priorities for the feedlot industry and the Australian government. Australia has a long tradition of high-quality management and production of feedlot cattle. The availability of veterinary medicines registered for use in cattle by the Australian Pesticides and Veterinary Medicines Authority (APVMA) provides essential support for the maintenance of the health and welfare of feedlot cattle. Amongst the most important veterinary medicines are the antimicrobial agents. The use of these invaluable medicines is undertaken under the professional guidance of feedlot veterinarians. This document presents a framework for antimicrobial stewardship, an approach to ensure the very best use of antimicrobial agents and it is designed for use by veterinarians, feedlot producers, the general public and Government. The antimicrobial stewardship framework document is currently published by MLA https://www.mla.com.au/globalassets/mla-corporate/research-and-development/program-areas/animal-health-welfare-and-biosecurity/mla_antimicrobial-stewardship-guidelines.pdf.

5.6 Antimicrobial stewardship guidelines for the Australian cattle feedlot industry.

From the antimicrobial stewardship framework document a concise and comprehensive set of guidelines for Antimicrobial Stewardship for the Australian cattle feedlot industry have been developed. The guidelines outline the five stewardship principles, termed the '5Rs', responsibility, review, reduce, refine and replace. These principles will help guide lot feeders towards best practice management use of antimicrobials and prevent overuse, which may contribute to the development of antimicrobial resistance.

The guidelines contain a step by step approach on how to develop an antimicrobial stewardship plan for a beef feedlot.

Effective implementation of an antimicrobial stewardship plan requires the following.

- Engage a veterinarian who has expertise in feedlot production and medicine.
- Ensure that a "Prescribed Drug List" and "Documented Treatment Protocol" has been developed by the veterinarian.
- Have an AMS team. Include the veterinarian and feedlot nutritionist on this team.
- Follow the 5Rs.
    - Develop a method to calculate the use of each antimicrobial
    - Develop a method of measuring compliance with the treatment protocol.
    - Use antimicrobials judiciously
    - Adopt preventative practices and review alternatives that will reduce the need to use medically important antimicrobials
    - Review the program regularly
- Develop a plan for monitoring the level of antimicrobial resistance in the feedlot including treatment success and antimicrobial response.
- Continue to uphold the integrity of grainfed beef through ongoing support of all integrity systems, especially LPA, NFAS, NLIS and NRS.

5.7 E-Learning Training Program

An important part of the Animal Health Management Program is the E-Learning training program. This is an online training program which is a modular format so that students can complete a course by completing the modules when it is suitable for them to do the training. The E-Learning program

has three courses, the “How to” series, the Practical Post Mortem of the Bovine course and the Antimicrobial Stewardship Series.

The format of each course is the same. There are a number of modules which contain information and explanation as text or pictures and video. At the end of each module there is a quiz which must be completed with all questions answered correctly before the student can move onto the next module.

Once all the modules have been completed correctly, the student will receive a certificate for having successfully completed that training course including answering all quiz questions correctly.

The three courses on the platform comprise:
1. How-To Series.
2. Practical Bovine Post Mortem Technique
3. Antimicrobial Stewardship Series

5.8 How To Series

This training course is a series of twelve modules covering tasks commonly performed by feedlot personnel.

The modules include:
1. Making a diagnosis – Identification.

This module has three sections
a) How to identify an animal
b) How to tag an animal
c) How to weigh an animal

2. Making a diagnosis – Taking rectal temperature.

How to take a rectal temperature.

3. Making a diagnosis – Listening (Auscultating) to lungs and rumen.

This module has four sections
a) How to auscultate lungs using manual (analogue) stethoscope
b) How to auscultate lungs using electronic stethoscope

There are recordings of lung sounds from animals with lung sounds of score 1, score 2, score 3, score 4 and score 5.

c) How to auscultate the rumen

4. Making a diagnosis - Rumen health

This module has four sections
a) How to measure rumen pH
b) How to collect a rumen fluid sample by stomach tube
c) How to perform a rumenocentesis
d) How to remove gas from the rumen via trocar and cannula

5. Making a diagnosis - Hoof health

This module has four sections
a) How to examine the hoof
b) How to pick up a hoof using rope and pully system

c) How to use a “Tip over table”
d) How to use a “standing hoof trimming crush”

6. How to take samples at Post Mortem.

This module has five sections
a) Collecting fresh tissue samples for bacteriology
b) Collecting fluids and exudates
c) How to take swabs for bacteriology
d) How to collect a fresh eye for toxicology
e) How to collect fixed or preserved tissues for histopathology

7. How to administer treatments by injection

This module has two sections
a) Needle know-how, needle selection, and needle care
b) Where to give injections

8. Methods of administrating treatments

This module has seven sections
a) How to give subcutaneous injections
b) How to give intramuscular injections
c) How to give intravenous injections
d) How to give injections at the base of the ear
e) How to implant
f) How to administer eye treatments
g) How to administer oral medications

9. How to read a product label and calculate a dose.

10. How to use in feed medication

This module has three sections
a) How to administer medication in a feed truck
b) How and why cleaning out a truck is important
c) How to top dress medication

11. How to stomach tube.

This module has two sections
a) How to collect fluid for rumen pH determination
b) How to administer medication orally.

12. Record keeping

This module has five sections
a) How to record treating one animal
b) How to record group treatment by injection
c) How to record group treatment by in-feed medication.
d) How to complete “Feed Medication” orders.
e) How to compile training records.

5.9 Practical Bovine Post Mortem Technique

This training course has twelve modules each with a set of assessment questions at the end of each module, and a section for links and resources.
The modules in the Practical Bovine Post Mortem Technique training course include:

1. Introduction
2. Equipment
3. The external examination
4. Opening the animal
5. The display stage
6. Examination of the head and neck
7. Examination of the heart and lungs
8. Examination of the gastrointestinal tract
9. The pelvic cavity
10. Liver, gall bladder and kidney
11. The stifle joint
12. The brain

5.10 Antimicrobial Stewardship Series

This training course has eleven modules each with a set of assessment questions at the end of each module, and a section for links and resources. Each module must be completed before moving onto the next module.
The modules in the Antimicrobial Stewardship series are:

1. Introduction
2. Critical importance of antibiotics
3. Antimicrobial resistance strategy
4. Defining antimicrobial stewardship
5. Responsibility on the feedlot
6. Reviewing use
7. Reducing use
8. Refining use
9. Antimicrobial resistance surveillance
10. Replacing antimicrobials
11. Implementation of an Antimicrobial stewardship program.

5.11 Training materials for AMS workshop

There are three possible methods of delivering the training material:
A. One day workshop;
B. Webinar;
C. “Soapbox” presentation delivered in two sessions.
Each of these methods will deliver the same material.

<u>Option A: One-day workshop</u>
This is the traditional one-day workshop. In this format, there would be a number of presentations which would provide background and information to the participants. The participants would be asked to develop their own AMS plan for their feedlot. This would be a “skeletal” outline which could be taken back to the feedlot for completion. Resource material would be provided. This would

include the AMS stewardship guidelines as well as login information for the on-line training materials. Time is provided for participants to commence their online training while in the workshop.
Workshop program.

8:30	Registration and Coffee	
9:00	Welcome and housekeeping	5 Mins
9:05	Background on AMS and ALFA priorities in AMS	15 Mins
9:20	What is Antimicrobial Resistance and Stewardship	30 Mins
9:50	Introductions to AMS Guidelines	20 Mins
10:10	Morning Tea	20 Mins
10:30	5R Framework of Antimicrobial Stewardship	30 Mins
11:00	How to develop an Antimicrobial Stewardship Plan for your feedlot	60 Mins
12:00	Lunch	45 Mins
12:45	Antimicrobial Stewardship and Grain fed beef integrity systems	30 Mins
1:15	Introduction to online learning tools	30 Mins
1:45	Online learning	60 Mins
2:45	What does this mean for your business?	30 Mins
3:15	Afternoon Tea and Close	

Option B: Using Webinars to deliver training
This is done with two webinars each of one hour.
Information is presented as a condensed version of the workshop, with questions that can be typed in as the Webinar proceeds.
Time is allocated to answer the questions at the end of the webinar.

Webinar 1:
1st Webinar Pre-reading material:
- Background on AMS and ALFA priorities in AMS
- What is Antimicrobial Resistance? (National and Global perspective)
- What is Antimicrobial Stewardship?

Webinar 1 program

11:00 am	Welcome and House keeping
11:05 am	Brief background on AMS & ALFA priorities in AMS
11.15 am	What is Antimicrobial Resistance and Stewardship?
11:25 am	ALFA/MLA work in the AMS space: Introducing the guidelines.
11:40 am	5R Framework of Antimicrobial Stewardship
11:50 am	Q&A
12:00 pm	Close

Webinar 2:
2nd Webinar pre-reading material:
- Antimicrobial Stewardship and Grain fed Beef Integrity Systems
- How to Develop an Antimicrobial Stewardship Plan for your feedlot

11:00 am	Welcome and Housekeeping
11:05 am	How to develop an Antimicrobial Stewardship Plan for your feedlot
11:15 am	Introduction to on-line learning tools – Demonstration
11:25 am	Antimicrobial Stewardship and Grain-fed beef integrity systems.
11:35 am	What does this mean for your business?
11:50 am	Q & A
12:00 pm	Close

Option C: Soapbox

Using “Soapbox” presentation technology to deliver the same material as in the Webinar. With this technology the presentations are pre-recorded.
Participants can view the presentations via a website.
Questions can be emailed to the presenter, who can answer the questions via another video presentation or by email.
Material presented in the “Soapbox” is identical to that delivered in the Webinars.

6 Conclusions/recommendations

An Animal Health Management Program has been produced for the Australian feedlot industry containing the following elements:

1. A situation review on antimicrobial resistance for the feedlot industry.
2. Survey of the Australian feedlot industry on antimicrobial use and practices.
3. Development of antimicrobial stewardship guidelines and framework for the Australian Cattle Feedlot Industry.
4. Literature review of alternatives to antibiotics for the prevention and treatment of commonly occurring feedlot diseases.
5. Development of eLearning courses for antimicrobial stewardship for the feedlot industry
6. Development of an outline and training materials on antimicrobial stewardship for the feedlot industry.

Meat & Livestock Australia and the Australian Lot Feeders’ Association are currently extending project outcomes to industry to promote antimicrobial stewardship.

7 Bibliography

The bibliography is presented as appropriate in each of the appendices to this report.

8 Appendix

The following appendices are attached to this report:

APPENDIX 1 ANTIMICROBIAL RESISTANCE SITUATION REVIEW
APPENDIX 2 MLA FEEDLOT QUESTIONNAIRE
APPENDIX 3 ALTERNATIVES TO ANTIBIOTICS FOR THE PREVENTION AND TREATMENT OF COMMONLY OCCURRING FEEDLOT DISEASES

Appendix 1.

SITUATION REVIEW

SUMMARY OF KEY POINTS

-Very few studies on AMR, both in foodborne, commensal and environmental organisms of potential public health significance as well as in bovine respiratory pathogens, have been conducted in the feedlot sector in Australia.

-Those that have been undertaken, especially concerning foodborne pathogens and commensals in healthy feedlot animals at slaughter have confirmed very low levels of AMR and importantly, no resistance to critically important antimicrobials.

-Resistance to third generation cephalosporins has recently been confirmed among multidrug-resistant *Salmonella* isolated from dairy cattle and calves in Australia.

-Despite much higher rates of antimicrobial use including greater use of critically important antimicrobials, AMR of public health significance is also not a major issue in feedlot cattle in North America compared to other livestock species, and very limited contamination of the carcass post slaughter has been reported.

-In several groundbreaking ecological studies conducted in North America, metagenomics analysis of environmental samples from feedlots has identified a large number of antimicrobial resistance mechanisms in the major bacterial groups, however most of the mechanisms engendered resistance to antimicrobials of low importance such as tetracyclines, rather than antimicrobials that are critical to human health.

-There are no recent Australian studies on the antimicrobial susceptibility profiles of *Mannheimia hemolytica* and *Pasteurella multocida* in feedlot cattle. In a recent study of *Histophilus somni* isolates from Australia, only a single isolate showed resistance to tetracycline.

-Outside the US, resistance among bovine respiratory disease pathogens is rare, but emerging resistance to tetracyclines and macrolides is documented.

-In US feedlots, the recent emergence of multidrug-resistant and almost pan-resistant isolates of the three main bovine respiratory disease pathogens is a cause for concern and a large risk to future animal health. All multidrug-resistant isolates contain a mobile genetic DNA element (known as an integrative-conjugative element or ICE) in their chromosome that contains multiple resistance genes with some isolates also showing resistance to fluoroquinolones. No isolate thus far has shown resistance to ceftiofur.

-The selection pressures that resulted in the emergence and spread of the ICE in US feedlot isolates are unknown, but mass medication for prophylaxis of bovine respiratory disease is one hypothesis being explored. However, in the only study conducted since the emergence of ICE-containing isolates, animals receiving mass medication were at no greater risk of harbouring MDR isolates than shaminoculated controls.

1. INTRODUCTION

Antimicrobial resistance (AMR) has been recognised as a global threat to human and animal health, with bodies such as the World Health Organisation (WHO) and the World Organisation for Animal Health (Office International des Epizooties [OIE]) calling on all nations to take urgent action to address the growing threat. Whilst the list of human use only drug classes will continue to grow as new drugs are discovered and developed, many of the traditional drug classes that are the mainstay of antimicrobial therapy are currently registered as both human and animal treatments (the so called “shared drug classes”). There are relatively few drug classes that are animal use only but some, such as the ionophores, are extremely important to feedlot cattle health. For the shared drug classes, harmonization of prescribing practices between human and animal health, reducing total antibiotic use as well as limiting the use of certain drug classes through adoption of prudent use guidelines, and regular monitoring of AMR through surveillance are designed with one goal in mind; to maintain the lifespan of shared drug classes whilst new classes are developed for the human health market. It is important that the Australian feedlot industry understands and adapts to the new environment, adopts antimicrobial stewardship principles that allow it to continue to treat and prevent bacterial infections and rumen dysbiosis in animals with confidence and actively encourages antimicrobial resistance surveillance to continue to assure the public that its products have the highest standards of safety with minimal impact on the environment.

Classification of use

Use of antimicrobials in human health is nearly always therapeutic (administration of an antimicrobial to a sick patient) with occasional prophylactic use (administration of an antimicrobial to prevent an infection). Use of antimicrobials in feedlot cattle can be classified as therapeutic, metaphylactic (treatment of sick and in contact animals to prevent spread of an infection), prophylactic and for growth promotion. Apart from the macrolide tylosin, which is undergoing review by the APVMA with potential label changes in the foreseeable future, the only antimicrobials approved for growth promotion in feedlot cattle represent classes of drug that are not used in humans and promote no cross-resistance to shared drug classes. Additionally, use of antimicrobials such as ionophores and virginiamycin to modulate rumen health is often erroneously confused by the medical profession and the wider public with growth promotion.

Guidelines on the use of critically important antimicrobials in Australian animals

In 2014, The Australian Veterinary Association (AVA) released a policy document on recommendations for the use of antimicrobials in animals that are critical to human health. The AVA, developed the first line, second line and third line approach principles in harmony with World Health Organization, the Food and Agriculture Organization, and the World Organization for Animal Health guidelines. In addition, the recent Australian Strategic and Technical Advisory Group on AMR (ASTAG) antimicrobial use in humans importance ratings (ASTAG 2015) were adopted. These guidelines are designed to promote conservation of the critically important third line or top shelf drug classes for serious, life-threatening or drug-resistant infections and should be incorporated within a therapeutic decision tree process (Figure 1).

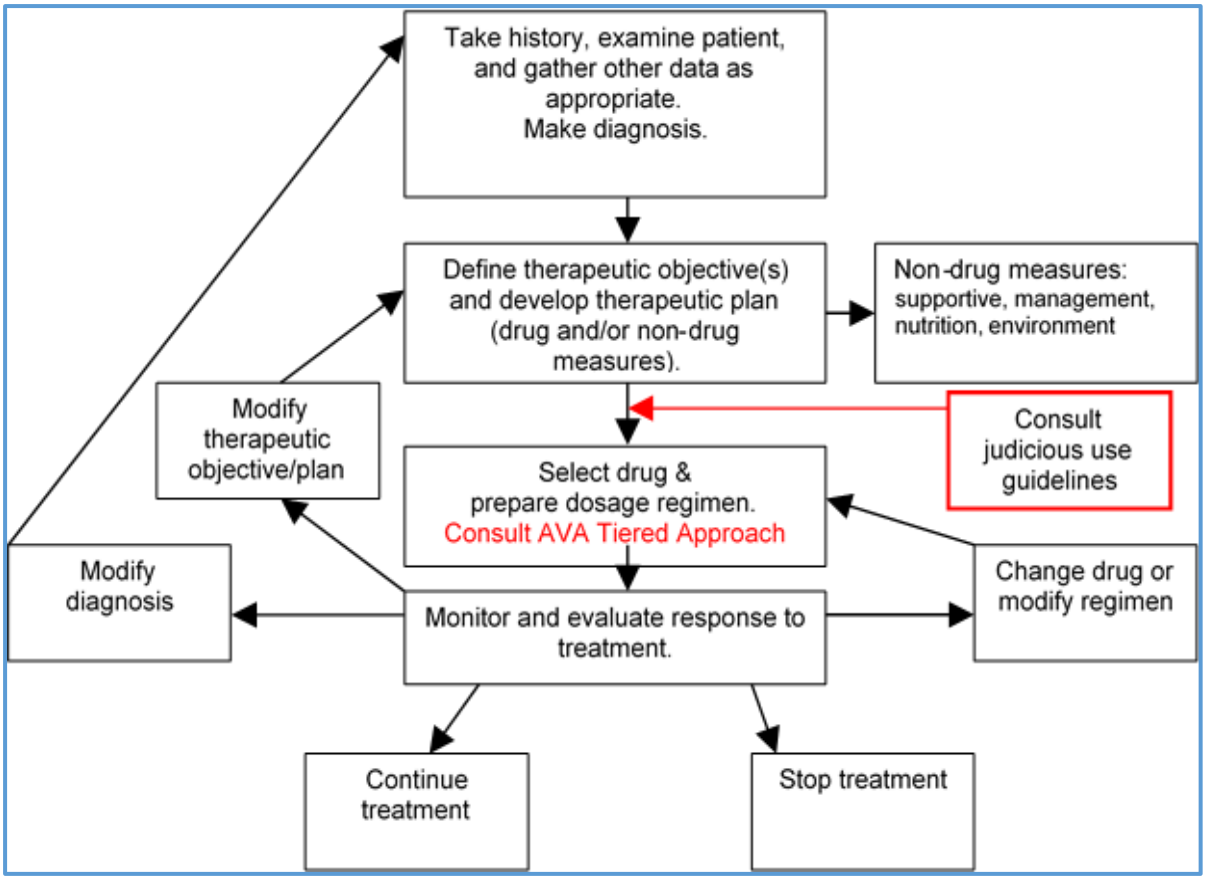

Figure 1: Antimicrobial and non-antimicrobial therapeutic selection decision making tree

Some examples of third line drug classes used in human medicine include fluoroquinolones, 3rd and 4th generation cephalosporins and carbapenems. According to the AVA guidelines, first line drugs can be used empirically in animals following clinical diagnosis of a bacterial infection. Second line use should be limited where possible to when susceptibility testing or clinical results have proven that first line antibiotics are not effective. Third line antimicrobials are for use as a last resort. They should be used only for serious or lifethreatening infections, when other options are unavailable and wherever possible only after susceptibility testing has been completed. Table 1 shows the classes of drug registered for use in cattle in Australia divided into the three categories. It should be noted that the fluoroquinolones (FQs), a class of broad spectrum antibiotics that are associated with AMR selection of great public health significance, are not included amongst the first, second and third line drugs. In fact Australia is the only country that has never registered fluoroquinolones for use in food-producing animals, a distinct public health advantage for our livestock industries. The only third line drug classes of relevance to the Australian feedlot industry are 3rd generation cephalosporins (ceftiofur) and streptogramins (virginiamycin). It is important to note that culture and susceptibility testing is often not possible in many cases of bacterial infection in feedlot cattle, with prompt selection and administration of the most appropriate antimicrobial required to treat infection and prevent disease progression according to treatment protocols.

Table 1: AVA guideline on the use of antimicrobials in cattle critical to human health.

First line	Second line	Third line	Not registered for therapeutic use in food animals
Ampicillin/ Amoxicillin	Amoxicillin-clavulanate (mastitis only)	Ceftiofur (3rd gen cephalosporin)	Fluoroquinolones Gentamicin
Erythromycin Oxytetracycline/ Chlortetracycline Sulphonamides Oleandomycin (mastitis) Tilmicosin Tylosin Penicillin Florfenicol Framycetin (topical) Neomycin Streptomycin (APVMA approved use only)	Cefuroxime (mastitis only) Cloxacillin (mastitis only) Apramycin Lincomycin Trimethoprim/ sulphonamide Tulathromycin	Polymyxin B (topical only) Virginiamycin (note this is the only product registered for use to prevent rumen acidosis. Even though they are listed as critically important, the importance of the streptogramin class to human medicine has been downplayed in recent years since the development of new drug classes).	

Acquisition, maintenance and transmission of antimicrobial resistance

The development of AMR in bacteria is complex. Bacteria can develop resistance by accumulating mutations in drug target genes located on the bacterial chromosome, but resistance most commonly occurs by direct acquisition of antimicrobial resistance genes (ARGs) that are encoded on plasmids, or less commonly by direct incorporation of ARGs into the bacterial chromosome. Plasmids are circular elements of DNA that replicate independently of the bacterial chromosome (Figure 2). They often contain accessory genes that may provide useful survival advantages in certain environments (such as in the presence of an antimicrobial) but are unnecessary for normal cellular growth and division. Plasmids that can be transferred both within and between bacterial species are termed F-plasmids and those F-plasmids that contain ARGs are called R-plasmids. R-plasmids have now evolved that contain ARGs encoding resistance to most major drug classes. If a bacterium acquires one of these multidrug-resistant (MDR) R-plasmids (the definition of multidrug resistance is resistance to one or more drugs in three or more classes), it can shift from fully susceptible to pan-resistant in a single genetic event. One of the major aims of restricting the use of third line antimicrobials in food-producing animals is to prevent the movement of newly evolved ARGs (such as those encoding resistance to third generation cephalosporins, fluoroquinolones or carbapenems) onto existing MDR plasmids present in livestock isolates that may encode resistance to first or second line drugs. Once an ARG encoding resistance to a third line drug is acquired, even if the drug is withdrawn from use, the ARG will be permanently located on the plasmid and use of first or second line drugs (to which the bacteria may also be resistant) will maintain the plasmid in the environment. This process is known as coselection.

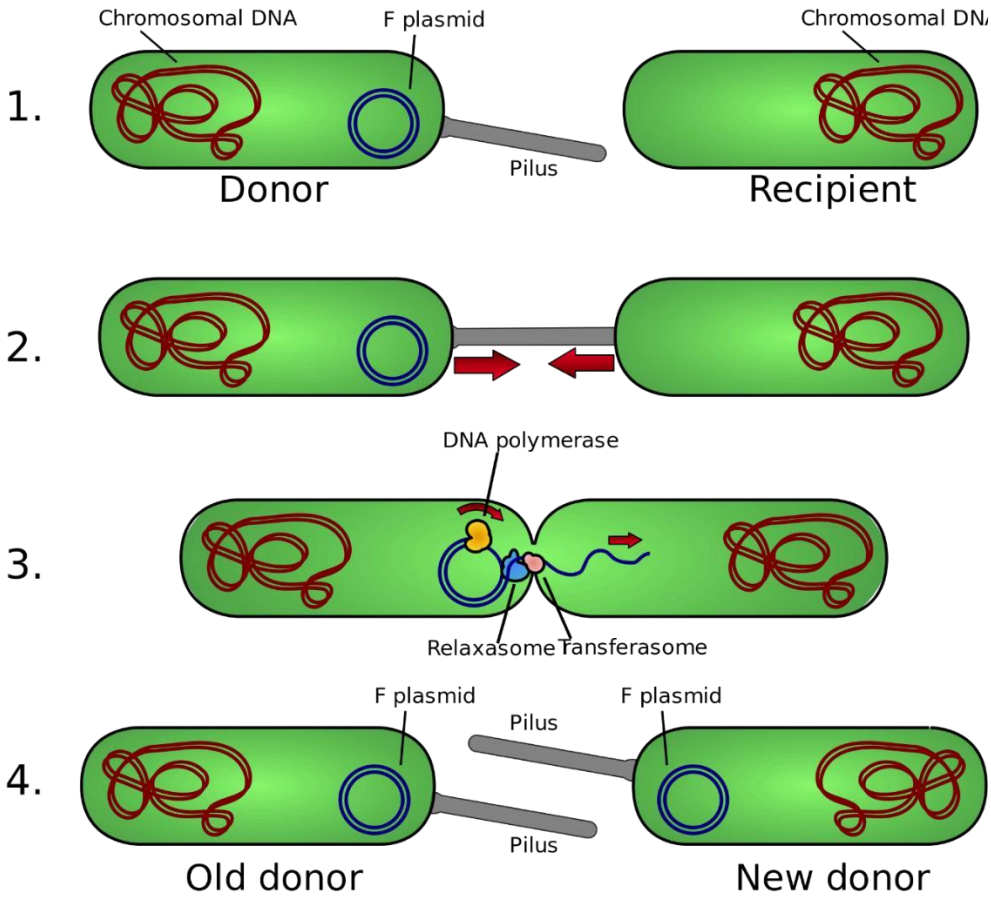

Figure 2: The process of F-plasmid (which can contain one or more AMR genes) transfer from a donor bacterium to a recipient bacterium (a process known as conjugation). 1. The donor bacterium expresses the sex pilus (these genes are located on the F-plasmid). 2. The sex pilus joins the donor bacterium to the recipient. 3. Replication of the plasmid occurs and is passed to the recipient bacterium. 4. The recipient bacterium now possesses the plasmid and the ability to transfer it to other bacteria.

A recent example of plasmid co-selection in Australia (Abraham et al 2016)

Figure 3 shows a genetic map of a bacterial plasmid identified in a MDR *Salmonella* isolate found in a domestic cat in Sydney. The cat was surrendered to a cat shelter and developed severe gastroenteritis following treatment of an upper respiratory tract infection with tetracycline. It is not known how the cat first acquired the *Salmonella* organism.

This plasmid encodes a beta-lactamase known as IMP-4 which provides resistance to carbapenems, regarded as one of the last line human therapies (but a class not registered for use in any veterinary species in Australia), but it also contains ARGs encoding resistance to nine other drug classes (shown in green, including to tetracycline). Even though there was no history of carbapenem use in the cat, the IMP-4 carbapenemase gene was co-selected by tetracycline use because it was located on the same plasmid as the *tetD* resistance gene. Of note, the plasmid also contains genes associated with resistance to heavy metals (shown in brown, the isolate was resistant to arsenic), suggesting that it is more than just antimicrobials that are responsible for the maintenance of R-plasmids in the environment. DNA sequence analysis showed that the plasmid is most closely related to R-plasmids previously identified in China. IMP-4 is the main carbapenemase identified in Gram-negative bacteria causing infections in humans in Australia, but

fortunately carbapenem resistance remains at a relatively low prevalence in hospitals and the community in this country, and importantly, has not been identified in bacteria isolated from feedlot cattle.

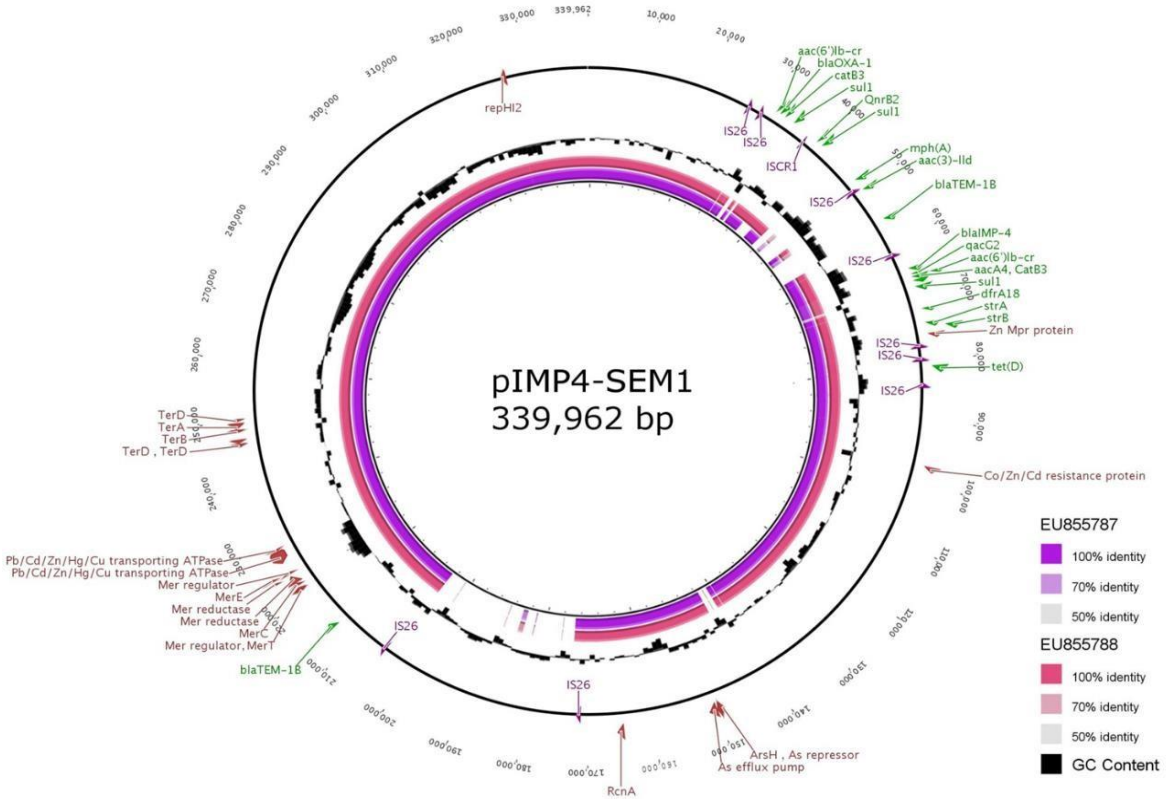

Figure 3: Genetic map of an IMP-4 encoding MDR plasmid identified in a sick cat at a Sydney cat shelter.

Antimicrobial resistance surveillance and the types of isolates investigated

The goal of antimicrobial resistance surveillance is to conduct the same survey at regular time points to establish baseline levels, trends and to determine the influence of intervention strategies in selected microorganisms. Human antimicrobial resistance surveillance focuses on the major pathogens where drug resistance is likely to be an issue, such as *Staphylococcus aureus, Enterococcus* spp., *Escherichia coli* and other Enterobacteriaceae, and *Neisseria gonorrhoea*. In animals, surveillance may focus on pathogenic clinical isolates obtained from veterinary diagnostic laboratories (both companion animal and foodproducing animal isolates) that may be animal-only pathogens (e.g. bovine respiratory disease isolates) or zoonotic organisms such as *S. aureus*, some *E. coli* and *Salmonella*. However, the majority of AMR surveillance activities throughout the world focus on micro-organisms that are present in the gastrointestinal tract of healthy food-producing animals at slaughter. These may be organisms causing foodborne infection in humans (*Salmonella* spp. and *Campylobacter* spp.), or the so-called "AMR indicator organisms" representing commensal Gram-negative (*E. coli*) and Gram-positive bacteria (*Enterococcus* spp.).

AMR testing

In AMR surveillance, resistance to panels of 12-16 antimicrobials are assessed according to internationally standardised methodologies to obtain the minimum inhibitory concentration (MIC) of the drug which is the lowest concentration required to inhibit the growth of the organism in a series of two-fold dilutions. The breakpoints used are recommended by standing committees such as the Clinical Laboratory Standards Institute (CLSI) and the European Committee on Antimicrobial

Susceptibility Testing (EUCAST) (the S breakpoint is the point at which an organism is classified as fully susceptible and the R breakpoint as "resistant" to the antimicrobial). However, there is currently much disagreement and uncertainty between CLSI and EUCAST breakpoints and the relative importance of clinical resistance breakpoints. Clinical resistance breakpoints relate to antimicrobial agent under review (especially the pharmacokinetics of the drug, which is influenced by the animal species) and the bacterial pathogen while the epidemiological cutoff values are designed to differentiate the fully susceptible "wild type" population from organisms that have reduced susceptibility. Not all antimicrobials have breakpoints established for all bacterial species, and the default is to apply human breakpoints to animal isolates, a practice that has many limitations.

Aim of the current review in the context of human health risks posed by feedlot use of antimicrobials

As a preamble to the development of an integrated antimicrobial stewardship programme for the Australian feedlot industry, this literature review aims to document any research conducted on AMR in the Australian feedlot industry, and any links to AMR in human or veterinary medicine, as well as making comparisons with studies conducted on AMR with other livestock sectors within Australia and in feedlots internationally. It will identify knowledge gaps and make recommendations on future directions. Table 2 lists the current threats to human health from AMR bacteria obtained from three different sources: USA (CDC 2013) which lists 18 threats, global (WHO 2014), which lists nine threats, and the recent Antimicrobial Use and Resistance in Australia (AURA) report, which lists 17 threats. A detailed examination would suggest that only two of these human threats, extended-spectrum beta-lactamase-producing Enterobacteriaceae and MDR non-typhoid *Salmonella* have any potential links to antimicrobial use in the Australian feedlot industry, specifically to use of the third generation cephalosporin, ceftiofur.

Table 2: Bacteria Drug Resistances and Feedlot Cattle Contribution to Public Health.

MICROBES WITH ANTIMICROBIAL RESISTANCE THREATENING PUBLIC HEALTH			
USA **CDC 2013** 18 threats	**GLOBAL** **WHO 2014** 9 threats	**AUSTRALIA** **AURA 2016** 17 threats	**FEEDLOT CATTLE LINK** 2 possible
URGENT THREATS (3)			
Clostridium difficile		Fluoroquinolone-resistant *Clostridium difficile*	No
Carbapenem-resistant Enterobacteriaceae (CRE)	*Klebsiella pneumoniae*, resistance to carbapenems	Enterobacteriaceae (mainly *Escherichia coli, Klebsiella* species and *Proteus mirabilis*)	No
Drug-resistant *Neisseria gonorrhoeae*	*Neisseria gonorrhoea*, resistant to 3GC	*Neisseria gonorrhoeae, Neisseria meningitides*	No
SERIOUS THREATS (12)			
Multidrug-resistant *Acinetobacter*		Multidrug-resistant *Acinetobacter*	No
Drug-resistant *Campylobacter*		FQR Campylobacter jejuni or C. coli	No

Fluconazole-resistant *Candida* (fungus)			No
Extended spectrum ßlactamase producing Enterobacteriaceae (ESBLs)	*Escherichia coli* & *Klebsiella pneumoniae*, resistant to 3GC	Extended spectrum ß-lactamase producing Enterobacteriaceae (ESBLs)	Possible
Vancomycin-resistant *Enterococcus* (VRE)		*Enterococcus* species	No
Multidrug-resistant *Pseudomonas aeruginosa*		Multidrug-resistant *Pseudomonas aeruginosa*	No
Drug-resistant nontyphoidal Salmonella	Non-typhoidal Salmonella, resistant to FQs	*Salmonella* species	Possible
MICROBES WITH ANTIMICROBIAL RESISTANCE THREATENING PUBLIC HEALTH			
		resistant to ampicillin, azithromycin, ceftriaxone/ cefotaxime, ciprofloxacin	
Drug-resistant *Salmonella* Typhi			No
Drug-resistant *Shigella*	*Shigella* species, resistant to FQs	*Shigella* species resistant to ampicillin, ciprofloxacin, trimethoprim/sulfamethoxazole, azithromycin	No
Methicillin-resistant Staphylococcus aureus (MRSA)	Methicillin-resistant *Staphylococcus aureus* (MRSA)	Methicillin-resistant *Staphylococcus aureus* (MRSA)	No
Drug-resistant *Streptococcus pneumoniae*	Drug-resistant *Streptococcus pneumonia*	Drug-resistant *Streptococcus pneumonia*	No
Drug-resistant tuberculosis		Drug-resistant tuberculosis	No
CONCERNING THREATS (3)			
Vancomycin-resistant *Staphylococcus aureus* (VRSA)		Vancomycin-resistant *Staphylococcus aureus* (VRSA)	No
Erythromycin-resistant Group A *Streptococcus*			No
Clindamycin-resistant Group B *Streptococcus*			No
	Escherichia coli, resistance to FQs	*Escherichia coli*, resistance to FQs	No
		Haemophilus influenzae type b ampicillin, ceftriaxone/ cefotaxime, ciprofloxacin	No

		Streptococcus agalactiae, pyogenes penicillin, erythromycin, clindamycin	No

The implications to human medicine of AMR arising from the use of antimicrobial agents in feedlot cattle practice is inextricably linked to the quality of use and the types of antimicrobials approved for use in feedlot cattle. While a survey of antimicrobial use will provide information on the degree of use of each antimicrobial agent, the antimicrobial formulary available for use is set out in Table 3 including the importance rating set out by ASTAG (2015).

Table 3: List of antimicrobial agents approved for use in cattle in Australia, and their relative importance to human health as assessed by ASTAG.

ANTIBACTERIAL AGENTS APPROVED FOR USE BY APVMA IN CATTLE		
ANTIBACTERIAL AGENT (n=37 registered for cattle)	**CLASS (n=16)**	**IMPORTANCE**
Novobiocin	Aminocoumarin	nhu
Apramycin	Aminoglycoside	low

ANTIBACTERIAL AGENTS APPROVED FOR USE BY APVMA IN CATTLE		
ANTIBACTERIAL AGENT (n=37 registered for cattle)	**CLASS (n=16)**	**IMPORTANCE**
Dihydrostreptomycin	Aminoglycoside	low
Framycetin[NF]	Aminoglycoside	low
Neomycin	Aminoglycoside	low
Streptomycin	Aminoglycoside	low
Trimethoprim[S]	Diaminopyrimidine	med
Flavophospholipol	Glycophospholipid	nhu
Lasalocid	Ionophore	nhu
Monensin	Ionophore	nhu
Narasin	Ionophore	nhu
Salinomycin	Ionophore	nhu
Lincomycin	Lincosamide	med
Erythromycin	Macrolide	low
Oleandomycin	Macrolide	low
Tilmicosin	Macrolide	low
Tulathromycin	Macrolide	low
Tylosin	Macrolide	low
Florfenicol	Phenicol	low
Bacitracin	Polypeptide i	low
Polymyxin B	Polypeptide ii	high

Virginiamycin	Streptogramin	high
Sulfadiazine^{T+}	Sulfonamide	low
Sulfadimidine$^{T+/-}$	Sulfonamide	low
Sulfadoxine^{T+}	Sulfonamide	low
Chlortetracycline	Tetracycline	low
Oxytetracycline	Tetracycline	low
Cephalonium	β lactam cephalosporin [1GC]	med
Cephapirin	β lactam cephalosporin [1GC]	med
Cefuroxime	β lactam cephalosporin [2GC]	med
Ceftiofur	β lactam cephalosporin [3GC]	high
Amoxicillin	β lactam penicillin	low
Ampicillin	β lactam penicillin	low
Cloxacillin	β lactam penicillin	med
Penethamate	β lactam penicillin	low
Penicillin (and salts)	β lactam penicillin	low
Clavulanic acid	β lactamase inhibitor	med
SUPERCRIPTS: S combination with a sulfonamide; T+/- with or without trimethoprim IMP importance for human medicine (ASTAG 2015) classified as low, medium and high; mhu minor human use; nhu no human use		

Critical Review of the Literature pertaining to Australia

A literature search of Pubmed and Web of Science was conducted using various combinations of the terms “antimicrobial resistance” and “feedlot”, “cattle”, “livestock” “pigs”, or “poultry”, and “Australia”. From the combined searches a total of 15 papers specifically pertaining to AMR in Australian beef feedlots or more widely, Australian cattle (including grazing cattle, dairy cattle and calves), or those involving Australianbased scientists that may have been conducted in feedlots overseas, were selected for detailed analysis. In addition, a search of publicly available government reports since the publication of the JETACAR report in 1999 was made. Studies pertaining to antimicrobial resistance surveys and/or surveillance activities were preferred over ecological studies and bacterial isolate genetic characterisation studies.

First AMR survey in bacteria isolated from healthy cattle in Australia

In November 2003, the then Department of Agriculture, Forestry and Fisheries (DAFF) commissioned a pilot study in direct response to the publication and acceptance of the 1999 JETACAR report. The study examined antimicrobial resistance in commensal *E. coli, Enterococcus* spp. and *Campylobacter jejuni* isolated from the gastrointestinal contents of Australian beef cattle, pigs and poultry following slaughter. No genotyping of the isolates was undertaken. Caecal specimens were obtained from healthy livestock at slaughter in Queensland, NSW, Victoria and South Australia. Greater than two hundred caecal specimens were obtained for each animal species. Culturing of samples was performed by Veterinary Diagnostic Laboratories in three States. Susceptibility testing was performed on over 500 *E. coli* and *Enterococcus* isolates and over 100 *Campylobacter* isolates. The study confirmed a low antimicrobial risk status for Australian food-producing animals. No enterococci were resistant to vancomycin, none of the *E. coli* isolates was resistant to third generation cephalosporins or fluoroquinolones, and no *Campylobacter*

isolates were resistant to fluoroquinolones. Multidrug resistance to classes of antimicrobial commonly used in each sector was identified, with pigs yielding the highest number of MDR phenotypes, followed by chickens and beef cattle. The study confirmed that existing resources within DAFF at the time could be adapted and equipped to assist with routine surveillance from processing plants.

Salmonella reference laboratory antimicrobial susceptibility testing

In Australia, antimicrobial susceptibility testing of *Salmonella* isolates of both human and non-human origin submitted to the Australian *Salmonella* Reference Centre (ASRC) commenced in May 2000 and was first reported in the 2001 annual report and last reported in the 2009 annual report.

The following table summarises the results of susceptibility testing of *Salmonella* isolates from Australian cattle, pigs and poultry submitted to the ASRC and summarised in annual reports for the period 2001 to 2009. The resistance of isolates to a panel of 11 antimicrobials was assessed. Antibiotic susceptibility testing was carried out by the Clinical Laboratory Standards Institute single break-point agar dilution method.

Importantly, no resistance to fluoroquinolones was reported with only a single sporadic isolate amongst the 1,977 isolates from cattle resistant to 3rd generation cephalosporins.

Table 4: Antimicrobial susceptibility testing of *Salmonella* isolates from cattle, pigs & broilers submitted to the Australian *Salmonella* Reference Centre. Ciprofloxacin (a fluoroquinolone) and ceftriaxone (a 3rd generation cephalosporin) resistance levels have remained at either zero or below 0.5% (shown in red).

Year	Species	Strains tested	Gen 4µg/ml	Kan 16µg/ml	Nal 16µg/ml	Chl 8µg/ml	Amp 8µg/ml	Tet 8µg/ml	Str 16µg/ml	Sul 256µg/ml	Tmp 4µg/ml	Cip 1&4µg/ml	Cef 1µg/ml
2001	Cattle	323	0.6	11.8	0.6[1]	5.3	17.0	15.8[1]	70.0[1]	22.0	17.0	0	NR
2001	Pig	104	0	9.6	10.6[1]	10.6	7.7	63.5[1]	75.0[1]	28.8	27.9	0	NR
2001	Poultry	1,076	0	1.1	0.5[1]	1.0	9.9	18.2[1]	74.3[1]	16.4	15.2	0	NR
2002	Cattle	299	1.0	3.3	0.3	1.7	4.7	3.0	36.5	8.4	6.4	0	0
2002	Pig	31	12.9	6.5	0	9.7	51.6	74.2	25.8	19.4	19.4	0	0
2002	Poultry	886	0	1.1	0	1.4	6.8	16.8	46.8	6.9	8.0	0	0
2003	Cattle	194	0	5.7	0	4.1	11.3	7.7	30.9	13.4	11.3	0	0
2003	Pig	218	24.3	17.4	1.4	6.9	40.4	50.0	34.9	33.0	16.1	0	0
2003	Poultry	963	0	1.3	0.1	0.7	9.6	22.5	31.8	4.8	5.1	0	0
2004	Cattle	167	0	8.4	0	3.6	18.6	10.2	42.5	19.2	16.8	0	0
2004	Pig	338	9.8	6.2	0	2.4	35.5	38.5	34.6	11.5	8.9	0	0
2004	Poultry	1,008	0.3	3.6	0	1.5	6.5	9.3	45.0	5.8	6.7	0	0
2005	Cattle	227	0.4	10.6	0.4	7.5	18.9	11.9	39.6	18.9	17.2	0	0
2005	Pig	77	7.8	9.1	1.3	5.2	31.2	37.7	40.3	23.4	16.9	0	0

2005	Poultry	1,925	0	3.0	0.2	0.9	6.2	10.3	47.0	7.6	7.3	0	0
2006	Cattle	230	0	7.0	0	3.9	11.3	9.1	15.6	13.9	10.0	0	0.4[#]
2006	Pig	111	5.4	10.8	3.6	7.2	55.9	51.4	45.0	45.0	17.1	0	0
2006	Poultry	1,618	0.1	2.1	0.2	0.4	5.0	13.0	32.9	6.2	5.9	0	0
2007	Cattle	237	0	5.1	0	3.0	7.6	7.6	11.0	10.1	8.0	0	0
2007	Pig	63	9.5	12.7	0	3.2	42.9	44.4	27.0	27.0	20.6	0	0
2007	Poultry	1,364	0	1.8	0.1	0.1	5.1	11.1	32.6	7.4	6.2	0	0.1[##]
2008	Cattle	170	4.1	7.6	0	4.7	10.0	10.0	15.3	13.5	10.0	0	0
2008	Pig	92	10.9	14.1	0	14.1	51.1	66.3	34.8	37.0	21.7	0	0
2008	Poultry	1,408	0	2.2	0	0.2	5.8	12.4	37.9	7.7	6.0	0	0
2009	Cattle	130	0	1.5	0	0.8	2.3	2.3	5.4	3.8	2.3	0	0
2009	Pig	69	17.4	23.2	0	14.5	82.6	88.4	76.8	71.0	20.3	0	0
2009	Poultry	1,475	0.3	1.4	0	0.4	6.0	10.4	31.4	6.6	4.3	0	0

Gen gentamicin [4]; Kan kanamycin [16]; Nal nalidixic acid [16]; Chl chloramphenicol [8]; Amp ampicillin [8]; Tet tetracycline [8]; Str streptomycin [16]; Sul sulfadiazine [256]; Tmp trimethoprim [4]; Cip ciprofloxacin [1 & 4 (all years) + 0.125 (2003-2009)]; Cef cefotaxime (3GC) [1]

[1] Antibiotic concentration in agar lower than other years. Nal 8µg/ml; Tet 4µg/ml and Str 4µg/ml

Single isolate of serovar Typhimurium phage type 4 resistant to cefotaxime, ampicillin and trimethoprim ## Single isolate of serovar Saintpaul resistant to cefotaxime and ampicillin

AMR in clinical *Salmonella* isolated from cattle

In 2014, Abraham et al. reported AMR profiles for a collection of 165 sequential *Salmonella* isolates from cases of salmonellosis in livestock (including 85 dairy cattle isolates and 21 beef cattle) obtained from diagnostic submissions by the NSW State Veterinary Diagnostic Laboratory (2007-2011). The most frequently detected serovars were Typhimurium (46.1%), Dublin (20.6%) and Bovismorbificans (13.3%). None of the isolates were resistant to critically important third line antimicrobials and rates of resistance to first and second line antimicrobials were generally low, with the highest frequency of resistance to sulphonamides (28.5%) and ampicillin (17%).

Largest point prevalence AMR study undertaken on Australian cattle to date (MLA funded)

In 2015, Barlow et al. reported on the AMR profiles of 800 *E. coli* and 217 *Salmonella* isolated from 910 healthy beef cattle, 290 dairy cattle, and 300 veal calf faecal samples collected at slaughter. Beef cattle were divided into grass or grain fed. Nearly all samples yielded *E. coli*, but *Salmonella* was only isolated from 14.4% of samples and was significantly more likely to be isolated from dairy cattle compared to beef cattle. Overall rates of resistance were low, and no resistance to third line third generation cephalosporins or fluoroquinolones was observed. MDR *Salmonella* were most likely to be recovered from grain-fed beef cattle; however, the resistance observed was to antimicrobials that would not be considered of critical or high importance to human medicine. Overall, the results corroborate previous Australian animal and retail food surveys that have revealed a low level of AMR, relatively small proportions of MDR isolates, and most importantly the maintenance of susceptibility to most antimicrobials of critical and high importance to human health. The most significant of these previous studies by Barlow and Gobius (2008), confirmed an extremely low incidence of AMR in *E. coli* isolated from retail beef in Australia. The results of AMR testing indicated that resistance to the majority of antimicrobials

tested was low (< 10%). In *E. coli* from poultry and pork the prevalence of AMR was ≥15% for ampicillin, streptomycin and tetracycline, in contrast to beef *E. coli* isolates where prevalence of resistance to these antimicrobials was ≤11%.

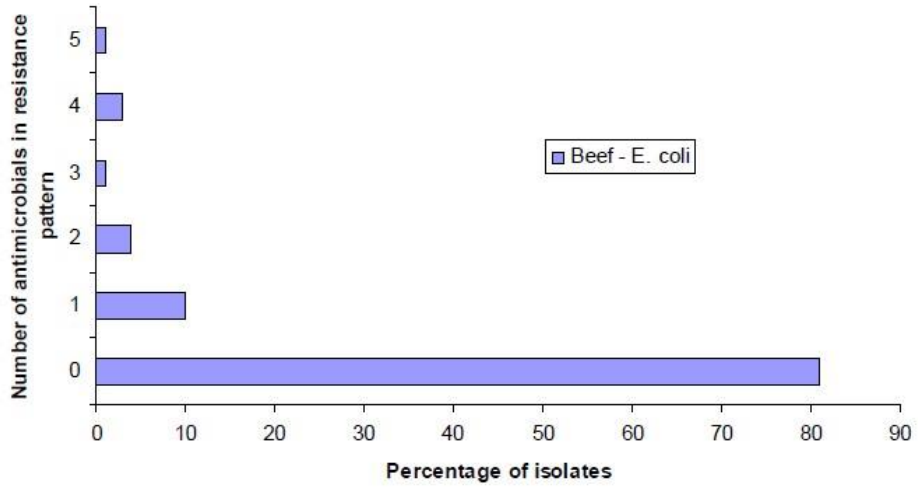

Figure 4. Drug susceptibility and resistance in *E. coli* from retail beef samples (n=100) (Barlow and Gobius, 2008).

The study by Barlow and Gobius (2008) of AMR in isolates of *E. coli* retail meat allowed a comparison of resistance profiles of *E. coli* between livestock species in Australia as well as comparisons with other significant cattle producing regions as summarised in Table 5.

TABLE 5: Antibacterial resistance (%) in *Escherichia coli* isolates from retail meat.

ANTIMICROBIAL AGENT	AUSTRALIA FSA 2008	AUSTRALIA FSA 2008	AUSTRALIA FSA 2008	CANADA CIPARS 4 Provinces 2013	EUROPE EFSA, ECDC 2013	USA NARMS 2012
	CATTLE	POULTRY	PIGS	CATTLE	CATTLE (for meat)	CATTLE
Amoxy-clav	**3**	**1**	**3.3**	**0-6.4**	-	**1.5**
Ampicillin, amoxicillin	**11**	**38**	**28.2**	**6.3-12.8**	9.5	**2.6**
Cefoxitin	**0**	**0**	**0**	**-**	-	**1.8**
Ceftiofur, cefotaxime	**0**	**0**	**0**	**0.9-8.5**	0.6	**0**
Chloramphenicol	**0**	**1**	**13**	**1.9-3.8**	5.5	**1.1**
Ciprofloxacin	**0**	**0**	**0**	**0-1.3**	1.9	**0**
Gentamicin	**0**	**4**	**1.1**	**0-6.4**	1.9	**0.7**
Kanamycin	**2**	**8**	**3.3**	**0-2.1**	-	**2.2**
Meropenem	**0**	**0**	**0**	**-**		**-**
Nalidixic acid	**0**	**0**	**0**	**0-2.1**	1.9	**1.5**
Streptomycin	**7**	**19**	**17.4**	**11.1-12.8**	15.1	**10.0**
Tetracycline	**7**	**47**	**44.5**	**19-24.1**	19.8	**22.1**
TMS	**5**	**22**	**13**	**1.3-2.1**	-	**0.4**

Notes: Bold entries represent isolates obtained from food, normal font represents isolates from gut. TMS trimethoprim + sulphonamide.

Zoetis funded survey identifies first report of third generation cephalosporin resistance and fluoroquinolone resistance in clinical *E. coli* isolates from Australian food-producing animals

In 2015, Abraham et al. reported on the preliminary characterisation of 324 clinical *E. coli* isolates obtained by veterinary laboratories throughout Australia from food-producing animals during the 2013 calendar year. The majority of isolates were most likely to be enterotoxigenic *E. coli* isolates. A total of 169 isolates were obtained from cattle sources (mainly dairy calves). A total of five isolates (two isolates from calves and three isolates from pigs) were resistant to 3rd generation cephalosporins, with one of the isolates from pigs also resistant to fluoroquinolones. One dairy cattle isolate from a liver sample possessed $bla_{\text{CTX-M-14}}$ and the second isolate from faeces possessed bla_{CMY}. Both these genes have been identified in human isolates both in Australia and overseas that are resistant to third-generation cephalosporins.

First reports of third generation cephalosporin-resistant *Salmonella* isolated from dairy cattle, dairy calves and humans in Australia

In 2011, in a study of AMR among 76 *Salmonella* isolates from dairy or dairy beef calves, Izzo et al. reported a single isolate (serovar Muenster) was resistant to ceftiofur based on disc diffusion susceptibility testing. This represents the first report of ceftiofur-resistant *Salmonella* in an Australian food-producing animal. In
2016, the Microbiological Diagnostic Unit at The University of Melbourne (which manages the national *Salmonella* reference laboratory) reported the first human cases of salmonellosis caused by multidrugresistant (MDR) *Salmonella enterica* serovar Typhimurium phage type DT44 that also expressed resistance to third generation cephalosporins (note: the definition of MDR is resistance to one of more individual drugs in three or more drug classes). In total, 31 third-generation cephalosporin-resistant isolates (13 human, 20 animal isolates) were identified from 2012-2016, each with a ceftriaxone MIC ≥64 µg/mL. Whole genome sequence analysis showed that the human and animal isolates were very closely related and 3rd generation cephalosporin resistance was mediated by a chromosomal CTX-M-9 beta-lactamase, with the isolates also exhibiting resistance to sulphonamide-trimethoprim, tetracycline, ampicillin, chloramphenicol and kanamycin. All the animal source isolates clustered in a single dairying region of Victoria.

First results from the federal Department of Agriculture sponsored AMR surveillance pilot project in Australian food-producing livestock species

In 2016, the first pilot project since the 2003 DAFF project was conducted by Australian Pork Limited in association with the federal Department of Agriculture. This survey was completed in October 2016 and analysed *E. coli, Salmonella, Campylobacter* and *Enterococcus* isolates from healthy pigs at slaughter throughout Australia and a report will be submitted to the Department for general release in November. However, preliminary data was presented at the recent Conference on Responsible Use of Antimicrobials in Agriculture*. E. coli* (n=203) showed the highest levels of resistance to ampicillin (60.1%), tetracycline
(68.5%), chloramphenicol (47.3%) and trimethoprim/sulfamethoxazole (34%). In comparison the *Salmonella* spp. (n=69) also showed the highest levels of resistance to ampicillin and tetracycline, albeit at much lower percentages (20.3% and 26.1%, respectively), and much lower levels of resistance to trimethoprim/sulfamethoxazole (11.6%) and chloramphenicol (7.2%). Low levels of resistance were observed in both species to amoxicillin/clavulanate (*E. coli* 9.4%; *Salmonella* spp. 2.9%) and gentamicin (*E. coli* 0.5%; *Salmonella* spp. 2.9%). No ceftiofur resistance was observed in either *E. coli* or *Salmonella* spp.
Two *E. coli* isolates had ciprofloxacin MICs of 8 mg/L which is above the wild-type ECOFF.
Four of eight isolates (50%) sent for whole genome sequence analysis, including the two ciprofloxacinresistant isolates belonged to broad host range commensal *E. coli* sequence type (ST) 10, which has been identified from both animals and in-contact humans in previous studies. This

confirms that even in the absence of antimicrobial resistance selection pressure (i.e. no fluoroquinolone use in food-producing animals in Australia), resistant isolates may still find their way into animal production systems, with the most likely route of entry being transmission from humans or wild migratory birds.

Antimicrobial resistance in Australian bovine respiratory disease isolates

There have been few studies undertaken in Australia specifically on AMR of bovine respiratory disease isolates. Stephens et al (1993) reported that 25/25 *Mannheimia haemolytica* and 24/25 *Pasteurella multocida* were fully susceptible to tilmicosin based on available breakpoints at the time. To the best of our knowledge no further published studies have been undertaken on *M. haemolytica*. Recently Goldspink et al (2014) examined the antimicrobial susceptibility patterns of 53 *Histophilus somni* isolates originating from feedlot cattle, with 51 isolates originating from bovine respiratory disease and one isolate each from cases of thrombotic meningioencephalitis and vaginitis. The isolates were tested for susceptibility to ceftiofur, enrofloxacin, florfenicol, tetracycline, tilmicosin and tulathromycin, using Clinical Laboratory Standards Institute (CLSI) disc diffusion and minimum inhibitory concentration testing. However tulathromycin MIC testing was only performed for 43 isolates. All isolates were susceptible to all six antimicrobial agents, except for a single tetracycline-resistant isolate. No other Australian studies were identified using the search terms “bovine respiratory disease”, “treatment failure” and “Australia”. Whilst this does not provide evidence that all bovine respiratory disease bacterial isolates from Australian feedlot cattle are pansusceptible as there are many reasons why an antimicrobial treatment could fail in addition to bacterial resistance, it does suggest that there has been relatively little incentive to further investigate isolate resistance profiles.

Comparison with international studies on AMR in feedlots.

Data from selected antimicrobial resistance surveillance programmes.

FRANCE

Surveillance of antimicrobial use and resistance in animals in France provides an interesting case study of significance to the Australian Feedlot Industry. The French National Observatory for Epidemiology of Bacterial Resistance to Antibiotics (ONERBA) centralises data from human and animal surveillance covering 17 surveillance networks. Created in 1997, ONERBA is an organization whose scientific and technical activities rely mainly on the networks for surveillance of resistance already established, only one of which (RESAPATH) is devoted to isolates obtained from animals. RESAPATH, operated by ANSES, the French Agency for Food, Environmental and Occupational Health & Safety, coordinates the voluntary contribution of antimicrobial susceptibility data from isolates from diseased food-producing animals and companion animals obtained by 63 public and private diagnostic laboratories distributed throughout the country.

RESAPATH is a key component of the EcoAntibio 2017 plan to combat antimicrobial resistance in animals. The EcoAntibio 2017 plan aims to reduce antimicrobial use in the veterinary sector by 25 per cent by 2017 by introducing/refining 40 broad measures divided into 5 axes. RESAPATH integrates disc diffusion antimicrobial susceptibility data obtained from participating private and public veterinary diagnostic laboratories distributed throughout France. Particular emphasis, however, is placed on *E. coli* isolates resistant to critically important classes of antimicrobial used in humans (3rd/4th generation cephalosporins and fluoroquinolones). ANSES monitors sales of antibiotics for veterinary use in France by compiling declarations from the point of sale. Data is cross-matched against declarations of turnover and prescriptions.

ANSES antimicrobial use data demonstrate an increase in consumption of antimicrobials of 27.9 per cent between 1999 and 2009, though data collected between 2009 and 2010 show a 12.2 per cent fall. However, during this time there has been a concomitant increase in the use of critically important antimicrobials (third and fourth generation cephalosporins and fluoroquinolones). RESAPATH have confirmed high rates of resistance to critical antimicrobials among *E. coli* isolates from cattle, horses and companion animals concomitant with increased availability and prescribing of these drugs. However they were able to demonstrate a drop in resistance frequency in their most recent report when EcoAntibio 2017 energies were focused on education and therapeutic guidelines for veterinarians.

CANADA

Similarly, the Canadian Integrated Program for Antimicrobial Resistance Surveillance (CIPARS) integrates antimicrobial use data in humans and animals with antimicrobial resistance surveillance in humans and food-producing animals (poultry, pigs and beef). Surveillance data has repeatedly shown the much lower public health risk associated with beef and pork compared to chicken. Surveillance data from CIPARS was instrumental in strengthening the understanding of how antimicrobial resistance in animals can have an adverse effect on public health. Presentation of human and animal data in an integrated fashion is useful for ensuring the animal surveillance and future interventions both have a focus on human health. Data concerning MDR *Salmonella* and *E. coli* were collected by CIPARS from 2004 onwards. The data demonstrated a link between an increasing frequency of detection of multi-drug resistant *Salmonella* Heidelberg in humans and the blanket use of ceftiofur in poultry production in parts of Canada, mainly as an *in ovo* injection to prevent *E. coli* infection in newly hatched chicks. Following a voluntary ban on this practice, rates of MDR *Salmonella* infection in humans dropped substantially.

The CIPARS report from 2013 (Government of Canada 2015) presents AMR results of surveillance of animal clinical isolates of *Salmonella*. Table 6 summarises the MIC data for cattle *Salmonella* isolates and demonstrates the high frequency of ceftiofur resistance, present in 42.7% of 248 isolates.

Table 6: MIC distribution for *Salmonella* isolated obtained from cattle in Canada.

Table 66. Distribution of minimum inhibitory concentrations among *Salmonella* from cattle

	Antimicrobial	n	Percentiles MIC 50	Percentiles MIC 90	%R	Distribution (%) of MICs (µg/mL) ≤0.015	0.03	0.06	0.12	0.25	0.5	1	2	4	8	16	32	64	128	256	>256
I	Amoxicillin-clavulanic acid	248	16	>32	44.0							36.7	1.6	1.2	9.3	7.3	15.7	28.2			
	Ceftiofur	248	1	>8	42.7				0.4		17.3	35.5	2.4	1.6	4.0	38.7					
	Ceftriaxone	248	≤0.25	32	43.5					56.0			0.4	4.4	0.4	6.9	25.4	5.6	0.8		
	Ciprofloxacin	248	≤0.015	0.12	0.0	60.5	28.2	0.8	2.4	4.4	3.6										
II	Ampicillin	248	>32	>32	61.3							35.1	2.8	0.8				61.3			
	Azithromycin	248	4	16	0.4							0.8	3.2	62.5	16.9	16.1	0.4				
	Cefoxitin	248	4	>32	41.5							10.9	31.0	10.5	5.2	0.8	5.6	35.9			
	Gentamicin	248	0.50	1	1.2					12.9	69.8	15.7	0.4			0.4	0.8				
	Kanamycin	248	≤8	>64	22.2										77.8				22.2		
	Nalidixic acid	248	4	>32	10.5							0.4	35.1	42.3	11.7			10.5			
	Streptomycin	248	>64	>64	58.5												41.5	5.6	52.8		
	Trimethoprim-sulfamethoxazole	248	0.25	0.50	5.6				49.6	35.5	5.6	3.6			5.6						
III	Chloramphenicol	248	>32	>32	54.4								1.6	16.5	27.0	0.4	0.4	54.0			
	Sulfisoxazole	248	>256	>256	66.1											6.5	21.4	6.0			66.1
	Tetracycline	248	>32	>32	64.5									35.5			4.4	60.1			
IV																					

USA

The United States National Antimicrobial Resistance Monitoring System (NARMS) provides comprehensive surveillance data on antimicrobial resistance in enteric bacteria from humans, retail meats and animals. An important feature of NARMS is that methodology in sampling and laboratories has been sufficiently stable since inception to allow for sound comparison of results between years thus demonstrating time-based trends in emergence of resistance. As well, the

laboratory methodology is comparable across the three arms of NARMS (humans, food and animals) which provides for a strong basis for 'one health' comparisons between these three sources. Very high rates of resistance to third generation cephalosporins have been a feature of *Salmonella* isolates from cattle sources, mainly driven by the emergence and spread of MDR *S.* Newport containing AmpC beta-lactamase, and more recently MDR *S.* Dublin and *S.* Kentucky (Figures 5 and 6), with decreased susceptibility to the fluoroquinolone ciprofloxacin a significant feature (FDA 2015; FDA 2016; Iwamoto et al 2016).

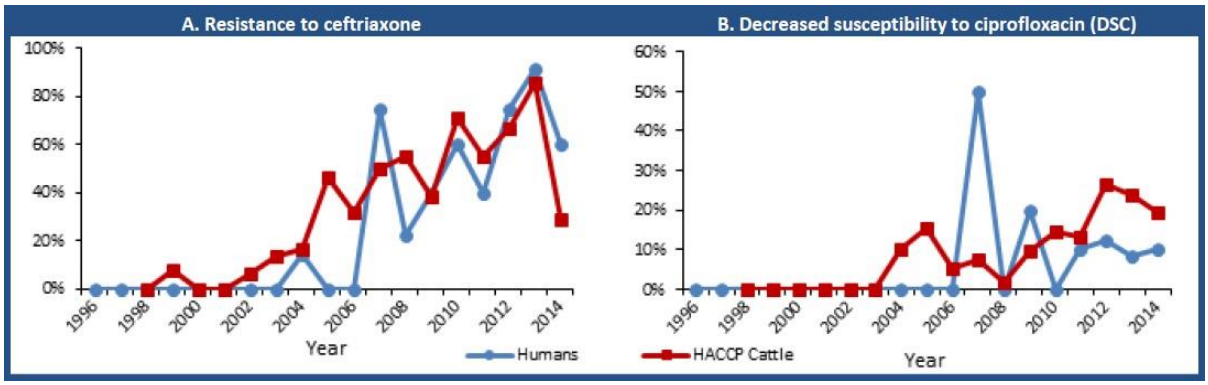

Figure 5: Percentage of *Salmonella* serotype Dublin isolates from humans and cattle with resistance to ceftriaxone and decreased susceptibility to ciprofloxacin, 1996-2014 (FDA 2016).

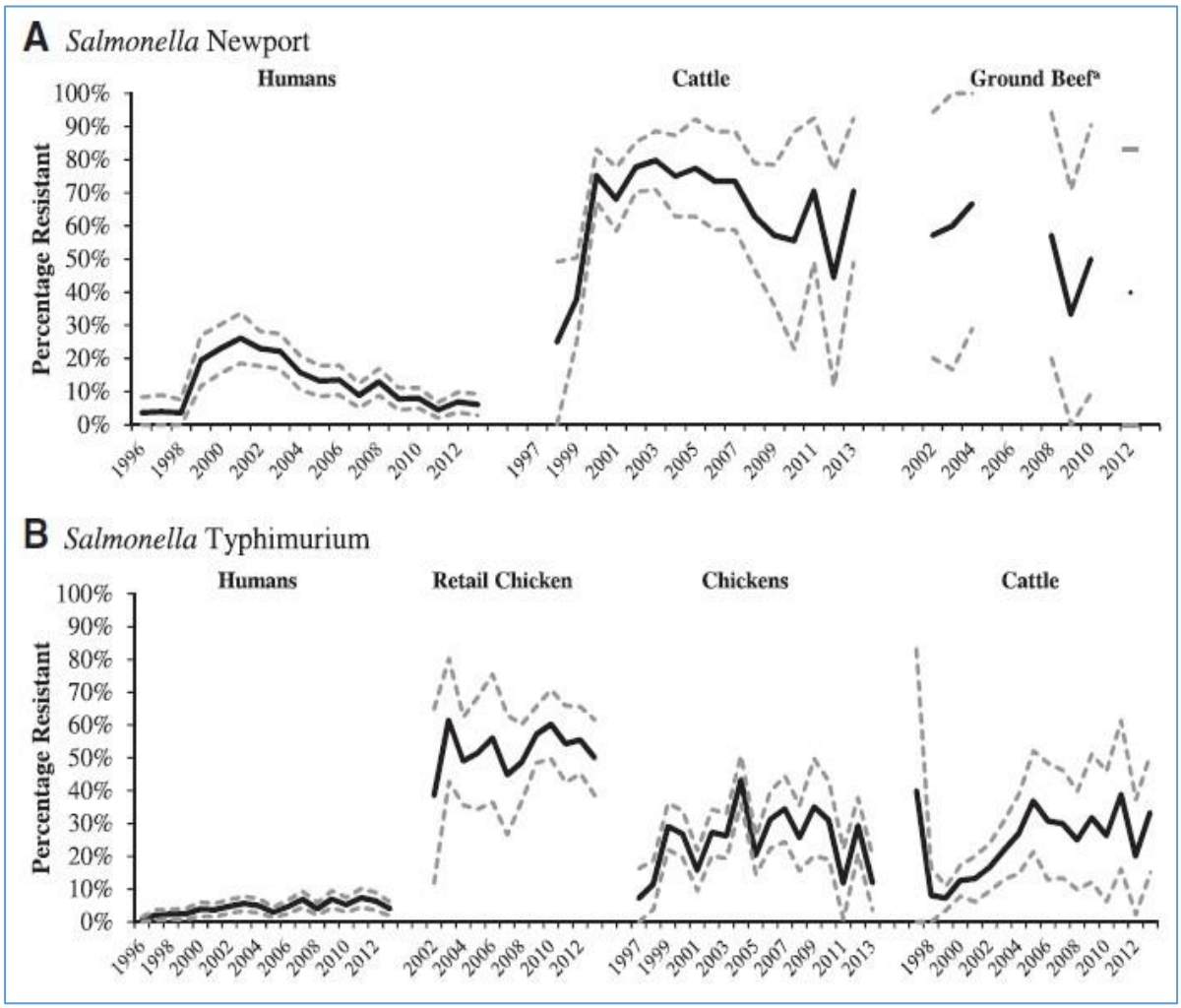

Figure 6: Agresti-Coull estimates and 95% confidence intervals for percentage resistant to ceftriaxone among *Salmonella* ser. Newport (A), and Typhimurium (B), by source, 1996–2013 [Iwamoto et al 2016].

A summary of the MIC distribution among *Salmonella* isolates obtained from ground beef over the period from 2002 to 2012 is set out in Table 7. Importantly, ceftiofur resistance was identified in 15.4% of isolates, though it should be noted that there were only 13 isolates available for testing.

Table 7: MIC distribution among *Salmonella* from ground beef (2002-2012) in NARMS surveys.

Table 12.3 MIC Distribution among *Salmonella* from Ground Beef, 2002-2012

Antimicrobial	Year (n)	%I[1]	%R[2]	[95% CI][3]	Distribution (%) of MICs (µg/ml)[4] 0.015	0.03	0.06	0.125	0.25	0.50	1	2	4	8	16	32	64	128	256	512	1024
Ceftiofur	2002 (9)	0.0	22.2	[2.8 - 60.0]						44.4	33.3				22.2						
	2003 (10)	0.0	40.0	[12.2 - 73.8]				30.0	30.0						40.0						
	2004 (14)	0.0	14.3	[1.8 - 42.8]						50.0	35.7				14.3						
	2005 (8)	0.0	0.0	[0.0 - 36.9]						37.5	62.5										
	2006 (19)	0.0	0.0	[0.0 - 17.6]						10.5	89.5										
	2007 (13)	0.0	0.0	[0.0 - 24.7]						30.8	61.5	7.7									
	2008 (24)	0.0	8.3	[1.0 - 27.0]						8.3	70.8	12.5			8.3						
	2009 (14)	0.0	14.3	[1.8 - 42.8]						14.3	71.4				14.3						
	2010 (7)	0.0	28.6	[3.7 - 71.0]							57.1	14.3			28.6						
	2011 (9)	0.0	11.1	[0.3 - 48.2]							88.9				11.1						
	2012 (13)	0.0	15.4	[1.9 - 45.4]						15.4	61.5	7.7		15.4							

NARMS surveillance also includes isolation and resistance testing of *Campylobacter jejuni* and *C. coli* from cattle and other livestock species. As presented in Figure 7, high ciprofloxacin resistance is present at high frequency, with 16% of beef cattle caecal isolates of *C. jejuni* and 62% of beef cattle isolates of *C. coli* resistant in 2014.

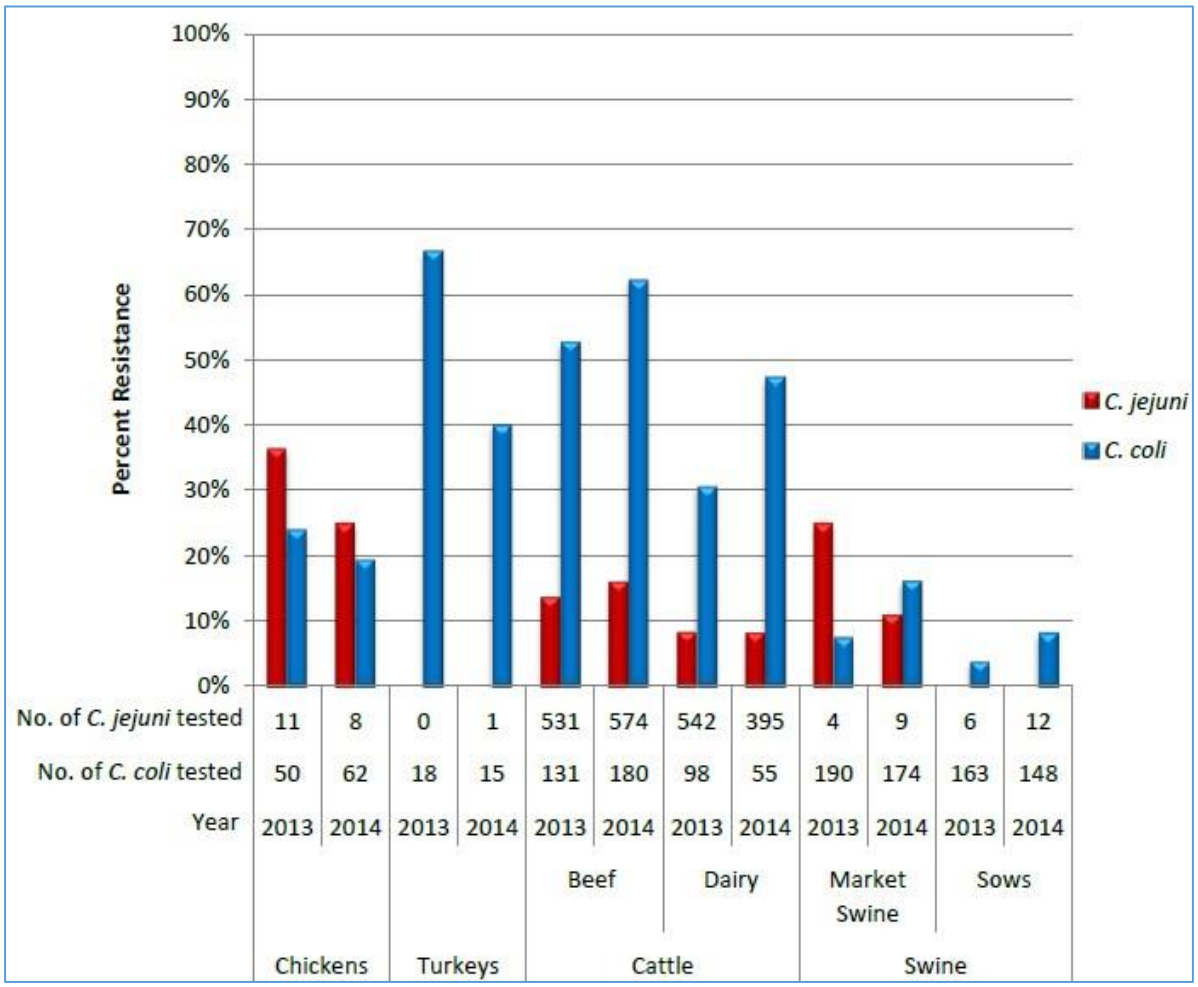

Figure 7. Ciprofloxacin resistance in *Campylobacter* from animal caeca 2013-2014 (FDA 2016).

Recent international published studies of note

A recent study by Dargatz et al (2016) found that from 2011 a low prevalence of *Salmonella* in the faeces of feedlot cattle in the USA. Whilst *Salmonella* was widely distributed, the overall prevalence in individual herds was less than 10%. Furthermore, the isolates were mainly all susceptible to antimicrobials with only some resistance to tetracycline and sulphonamides detected. Given that the rate of antimicrobial use in US feedlots is comparatively higher than it is in Australia, this study suggests a comparatively low impact to public health. It is important to note that fluoroquinolones can be used in Nth American feedlots for bovine respiratory disease.

In the first study of its kind, Noyes et al (2016b) identified the AMR mechanisms present in total microbial communities present in soil, effluent and wastewater of dairies, feedlots and ranch operations in North America using metagenomics (so called “resistome profiling”). Metagenomics is the simultaneous sequencing and assembly of multiple genomes from bacteria in ecological samples. A total of 34 different resistance mechanisms were identified with tetracycline resistance mechanisms identified most frequently in all cattle production systems. Clear differences were identified between feedlots and dairies, with ranches having comparatively fewer instances of resistance. Additionally, the resistome of faecal samples, wastewater and soil all differed. Other than providing baseline data, it is difficult to quantify risks to public health from such studies. In a cohort study of animals progressing through a beef feedlot from entry to slaughter, Noyes et al. (2016a) also used “resistome profiling” to confirm that AMR genes were generally absent in feedlot beef products, suggesting that slaughter interventions may reduce the risk of transmission to humans. They concluded that the risk of antimicrobial resistance genes in the environment may represent a greater risk than the food supply with respect to antimicrobial use in feedlots, but generally, resistance mechanisms were dominated by genes imparting resistance to low importance drugs.

Smith et al, (2016) found no evidence of fluoroquinolone resistance in *Salmonella* isolated from beef feedlots in the USA using fluoroquinolones as the primary therapeutic antimicrobial to treat BRD in these feedlot populations. In a large trial of US feedlot cattle sampled at entry to the feedlot and then during the feeding period, Benedict et al (2015) found modest increases in *E. coli* resistance to tetracycline, sulphonamide and streptomycin only, rather than any critically important antimicrobial.

In a response to concerns about antimicrobial-resistant pathogens being transferred from feedlot cattle through meat to consumers, Schmidt et al. (2015) showed that the rate of contamination through the slaughter process in US feedlot cattle was extremely low; nevertheless, *E. coli* that was resistant to thirdgeneration cephalosporins were detected in 0.5% of carcasses. However, only two of 525 *E. coli* isolates characterised could be regarded as extraintestinal pathogens of humans, indicating that products from fed cattle are not a significant source of bacteria causing human urinary tract infections and sepsis.

Collectively, these data would suggest that even in a country with access to more critically important drug classes and higher use patterns, the risk of AMR of significance to human health being generated by antimicrobial use in beef feedlots remains low. It is therefore likely to be even lower in Australia and the limited studies that have been conducted to date confirm this minimal risk. Figure 8 (Hurd 2006) summarises the series of events that have to occur in order for an antimicrobial administered to animals in a feedlot scenario resulting in a human developing a bacterial infection that is resistant to that antimicrobial from consuming product from that animal (using macrolides as the paradigm).

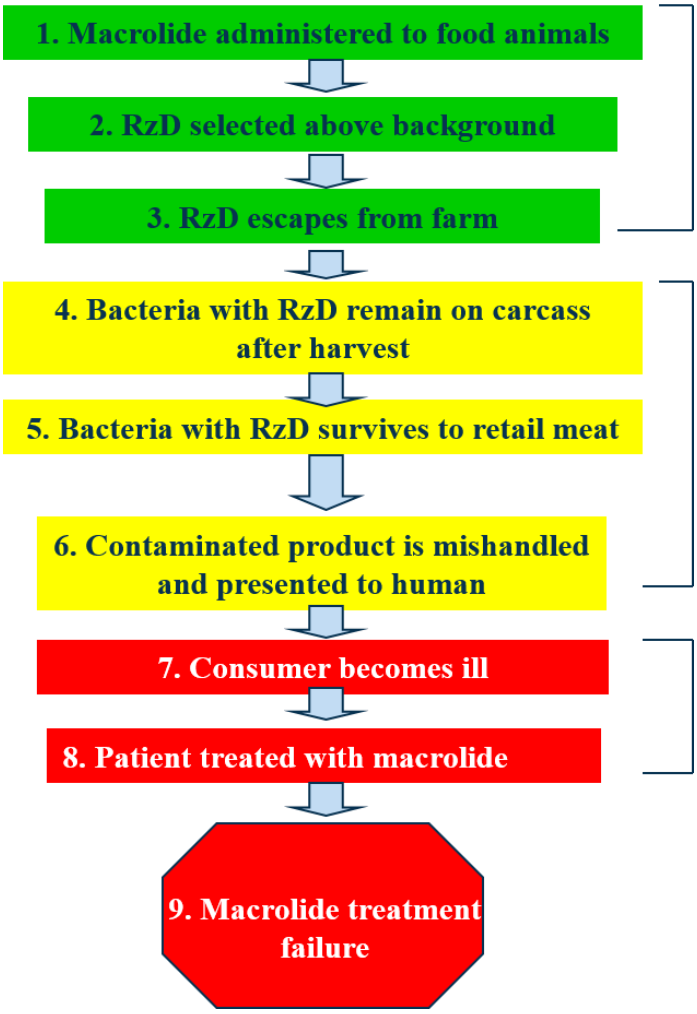

Figure 8: The necessary events required for a MDR bacteria to move through the food chain and cause a clinical infection in a consumer. (RzD = resistant bacteria or resistance determinants)

International studies documenting AMR in bovine respiratory disease pathogens

Lubbers and Turnidge (2015) provide an excellent review of antimicrobial susceptibility testing and bovine respiratory disease and the challenges posed by this particular veterinary setting in terms of accurate diagnostic sampling to yield relevant isolates. They concluded, that rather than be guided by individual culture and susceptibility results, bovine feedlot practitioners should develop "cumulative antibiograms" of their herds (obtaining a number of post-mortem isolates during peak times when bovine respiratory disease is prevalent [in particular from treatment failures] and comparing susceptibility profiles across years to detect emerging resistance).

Garch et al. (2016) monitored antimicrobial susceptibility in bovine respiratory pathogens (*Mannheimia, Histophilus* and *Pasteurella*) obtained from European cattle between 2009-2012. They concluded that the majority of pathogens remained susceptible to registered drugs apart from a low to moderate level of resistance to tetracycline (3.0-12.0%) and emerging resistance to macrolides (0–4.0%).

In a risk factors study in Canadian feedlots, Noyes et al (2015) concluded that the identification of resistant isolates among bovine respiratory disease pathogens was relatively rare. Nevertheless, exposure to antimicrobial drugs in pen mates was associated with increased odds of recovering multidrug-resistant *M. haemolytica*.

Dedonder and Apley (2015) reviewed the literature documenting resistance in bovine respiratory pathogens in the US and identified 16 articles where resistance was reported. One of the most significant publications by Watts et al (1994) conducted susceptibility testing of 888 bovine respiratory disease isolates. They documented confirmed resistance to tilmicosin, a relatively newly introduced antimicrobial at the time and the possible presence of cross-resistance among the macrolide class of agents. Studies between 1994 and 2008 confirmed the trend of low levels of cross-resistance among the macrolides, fluctuating levels of resistance to tetracycline, but uniform susceptibility to florfenicol, ceftiofur and fluoroquinolones. Lubbers and Hanzlicek (2013) examined the prevalence of resistance among BRD pathogens from submissions to the Kansas State University Veterinary Diagnostic Laboratory and identified an alarming trend of increasing MDR between 2009 (42% of isolates) and 2011 (63% of isolates). By 2011, a total of 25% of the isolates were resistant to four of six antimicrobials, with only ceftiofur and florfenicol showing uniform susceptibility. The genes associated with macrolide resistance in BRD isolates have been identified as *erm, msr* and *mph*.

Identification of integrative-conjugative elements containing multiple antimicrobial resistance gene families in bovine respiratory disease isolates

In 2012, the first integrative-conjugative element (ICE) ICEPmu1 was identified in a *P. multocida* isolate from a case of bovine respiratory disease (Michael et al., 2012a,b). ICEs are mobile DNA segments that can accumulate multiple antimicrobial resistance genes and integrate into the bacterial chromosome at very specific sites. ICEPmu1 contains 12 antimicrobial resistance genes including genes imparting resistance to macrolides, florfenicol, aminoglycosides, sulphonamides, amoxycillin and tetracyclines and similar ICEs have subsequently been identified in *M. haemolytica* and *H. somni* indicating cross-species transfer (Figure 8). Isolates containing these ICEs are often resistant to all drugs registered for the treatment of bovine respiratory disease (in North America) except ceftiofur and fluoroquinolones. This presents a very concerning trend in beef feedlot medicine, as it is possible for an isolate to move from full susceptibility to resistant to nearly all possible treatment choices in a single genetic event. In a very recent study, Klima et al (2014) investigated mortalities in feedlots in Canada, Texas and Nebraska and concluded that over one third of the US isolates were resistant to more than seven antimicrobial classes, including aminoglycosides, penicillins, fluoroquinolones, lincosamides, macrolides, pleuromutilins, and tetracyclines. Nearly all these isolates possessed an ICE, however the isolates were not clonally related, indicating movement of similar ICEs among distinct isolates rather than dominance of one particular sub-type.

Dedonder and Apley (2015) proposed that further investigation of the possible selection pressures that could have led to the emergence and spread of ICEs within beef feedlot isolates in the US was required. In the only recently published study following their review article, Dedonder et al (2016) confirmed that there was no difference between cattle previously treated with gamithromycin by mass medication for prevention of bovine respiratory disease or sham-inoculated controls with respect to yielding gamithromycin-resistant isolates.

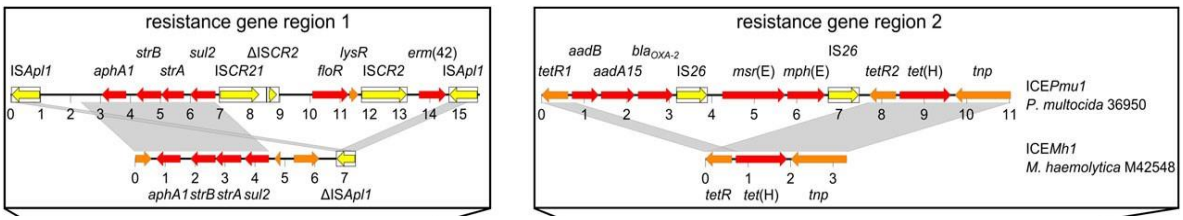

Figure 9: Similar resistance encoding regions identified in ICE elements from bovine respiratory disease pathogens *P. multocida* and *M. haemolytica* (Eidam et al. 2015).

Causes of apparent antimicrobial treatment failure in beef feedlots

Whenever the response to treatment of cattle with confirmed or presumed bacterial infections is less than expected it is important to investigate to determine the cause. It is only when the actual cause or likely causes is identified that appropriate changes to treatment can be made. A systematic investigation of apparent treatment failure is essential as there are many possible causes of apparent treatment failure as set out in the following table. Resistance of the causal agent to antimicrobials is just one of seven microbial factors that require consideration.

Table: 8: Twelve major factors associated with apparent treatment failure in cattle
Diagnosis ○ Condition not of bacterial origin (non-infectious (eg plant poisoning), other infectious (eg fungal, viral or protozoal infection)
Therapeutic goals ○ Unrealistic objective (bacterial eradication vs disease control)
Pathophysiology ○ Progression of underlying disease ○ Poor management of mixed infection (eg mixed aerobic and anaerobic infection)
Host factors ○ Predisposing factors uncorrected ○ Impaired immune function (eg failure of passive transfer of colostral immunoglobulins) ○ Nutritional deficits
Pharmaceutical factors ○ Substandard product (expired, inappropriate storage)
Treatment ○ Poor compliance (eg treatments not administered) ○ Misadministration (eg animal avoided treatment, oral dosage regurgitated, injection misdirected)
Pharmacology ○ Inappropriate drug selection ○ Inappropriate dosage regimen (inadequate dose rate, route, frequency, duration) ○ Pharmacokinetic issues (esp changes in absorption, distribution and clearance) ○ Impaired perfusion and penetration (blood brain barrier, abscess, oedema, swollen milk ducts, etc) ○ Interaction with concurrent medication
Supportive therapy ○ Omission of concurrent supportive measures (nutrition, hydration, nursing, abscess drainage, sequestrum removal)

Microbial factors ○ Toxin elaboration ○ Antimicrobial drug resistance (AMR) ○ Reinfection ○ Bacterial dormancy/persistence (eg non growth phase) ○ Bacterial L-forms ○ Phenotypic tolerance (eg small colony variants)
Table: 8: Twelve major factors associated with apparent treatment failure in cattle
○ Inoculum effects - dense bacterial loads in infected tissue ○ Biofilm formation ○ Superinfection (bacteria or fungal) ○ Poor correlation of in vitro susceptibility and clinical outcome (eg in vitro rapid growth vs slower growth in milk)
Epidemiology ○ External bacterial challenge continues unabated
Toxicology ○ Apparent failure due to adverse drug reaction, not infection control failure
Investigation Failure ○ Inappropriate samples collected ○ Non-representative animal(s) investigated (eg post mortem of untreated animal)

While it might be tempting to conclude that apparent treatment failure is due to AMR this cause is infrequently found following investigation. If the cause is incorrectly assumed to be due to the presence of AMR in the implicated pathogens then treatment choices will be modified unnecessarily with the possible use of antibacterial agents that are less appropriate or may have increased likelihood of selecting AMR – both unintended adverse consequences.

Conclusions, knowledge gaps and implications for the Australian feedlot industry.

Risk of antimicrobial resistance resulting from antimicrobial use in the Australian beef feedlot industry to harm public health

Ceftiofur is used in Australian feedlots for the treatment of respiratory disease in individual animals. The accompanying MLA antimicrobial use survey with this report will give an indirect measurement of how ceftiofur is used and how much it is used in comparison to other drug classes in Australian feedlots. This will provide important information for a national antimicrobial stewardship programme for beef feedlots in terms of the potential, however small, for public health impact resulting from the use of a criticially important third line treatment.

Currently, the AMR status of the Australian feedlot beef herd is negligible on the basis of a single point prevalence survey and comparable to AMR status of grass fed animals apart from having an increased prevalence of tetracycline resistance in *E. coli.*

Isolation frequency and associated risks of MDR *Salmonella* from healthy grain fed animals appears to be much less than the corresponding risk for cull dairy cows in Australia, according to the latest data.

The emergence of ceftiofur resistance in MDR *Salmonella* isolates from dairy cattle and calves in Australia and the detection of small numbers of similar isolates causing human infections are certainly causes for concern. Third generation cephalosporins such as ceftriaxone are used to treat cases of severe salmonellosis in children due to the fact that fluoroquinolones cannot be used in growing individuals.

Ceftiofur has traditionally been the most expensive of the registered drugs for bovine respiratory disease, but is now off patent and a number of cheaper formulations have become available. These human cases of third generation cephalosporin-resistant *Salmonella* come amid increasing anecdotal reports of off-label use of ceftiofur for foot diseases in dairy cattle due to the nil withholding period for milk and its documented use in calves with scours. Whilst the risk of such MDR strains emerging in feedlot cattle in Australia is extremely small, developing clear stewardship guidelines governing the use of ceftiofur in Australian feedlots would be a pragmatic step.

Regular surveillance of antimicrobial resistance as exemplified in the Barlow study, will confirm the low risk to human health from the consumption of feedlot beef products.

Risk of antimicrobial resistance resulting from antimicrobial use in the Australian beef feedlot industry to harm animal health

Whilst the absence of evidence is not evidence of absence, antimicrobial resistance amongst bovine respiratory disease pathogens does not appear to be a major issue facing the Australian feedlot industry.

Low to moderate resistance to tetracyclines and emerging macrolide resistance has been identified in Canadian and European bovine respiratory disease isolates.

The emergence of almost pan-drug-resistant isolates of *Mannheimia haemolytica, Pasteurella multocida* and *Histophilis somni* in US feedlots due to the emergence and horizontal spread of ICEs among isolates is a major cause for concern. Until reasons underlying the emergence and spread of ICE-bearing strains are determined, it would be prudent for the Australian beef feedlot industry to commence an antimicrobial resistance surveillance plan based on susceptibility testing of isolates so that any increased frequency of resistance to key registered drugs is detected early, especially if increased frequency of treatment failures is reported. However, passive surveillance through collecting and testing isolates submitted to Veterinary Diagnostic Laboratories may not be sufficient due to low numbers of submissions and active surveillance undertaken each year during bovine respiratory disease season may be more beneficial.

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Appendix 2. MLA Feedlot Questionnaire

37. MLA feedlot questionnaire

I am calling on behalf of the University of Adelaide regarding a MLA funded and ALFA supported project on Antibiotic Stewardship for the Australian Beef Feedlot Industry. You will have recently received some information in the mail consisting of a copy of the survey, a letter of support from MLA and ALFA and some information on antibiotic use and antibiotic resistance. The survey will be conducted by telephone. You will be asked a series of questions on the use of certain antibiotics and storage and access of veterinary drugs at your feedlot. Results from the survey will be used to develop an antibiotic stewardship training program for the Australian beef feedlot industry. Your property details will be kept strictly confidential. A confidentiality code has been assigned to all respondents so that none of the researchers analysing the data survey responses will know who you are. Your answers will be entered into a web-based application, starting with your assigned confidentiality code. The survey may take up to 30 minutes to complete. Are you able to participate now? Alternatively we can make a time that is more convenient for you to complete the survey. Let's begin the survey.

Enter confidentiality code here _____

38. Background questions

Select the feedlot size category that best describes your business

- ❍ < 3,000 animals
- ❍ 3,000-10,000 animals
- ❍ > 10,000 animals
- ❍ Unanswered

In the past 12 months, how many animals in total were sold from this feedlot?

- ❍ < 10,000
- ❍ 10,000 - 20,000 ❍ 20,000-30,000
- ❍ 30,000 - 40,000
- ❍ >40,000
- ❍ Unanswered

In the past 12 months, what is the average time an animal will spend in the feedlot?

- ❍ <80 days
- ❍ 80-150 days
- ❍ > 150 days
- ❍ unanswered

In the past 12 months, what percentage of total animals in the feedlot were 'pulled' for treatment?

_____

Tylosin

Have you used tylosin by injection (trade names Bilosin, Tylan, Tylopharm) in the past 12 months?

- ❍ Yes
- ❍ No
- ❍ don't know
- ❍ unanswered

SKIP LOGIC HERE – IF RESPONDENT ANSWERED YES THEY PROCEED TO NEXT QUESTION. IF RESPODENT ANSWERED 'NO', 'DON'T KNOW' OR 'UNANSWERED' THEY SKIP TO NEXT BLOCK

What percentage of 'pulled' animals were given injectable tylosin in the past 12 months?

_____

Nominate the purpose/s of using injectable tylosin (individual treatment, mass treatment response, timed or scheduled treatment) and the disease syndromes treated

	Individual	mass treatment	timed/ scheduled treatment
Respiratory (e.g. laryngitis, pneumonia)	❑	❑	❑
Gut (e.g. acidosis, blood scours, scours, rumen health)	❑	❑	❑
Musculoskeletal (lameness, footrot, swollen joints/ legs, foot abscess, lumpy jaw, wooden tongue)	❑	❑	❑
neurological (e.g. PEM/TEME, dropped ear, circling, pink eye)	❑	❑	❑
Urogenital (e.g. prolapsed prepuce, calving, waterbelly, castration)	❑	❑	❑
Other (e.g. tick fever, honker)	❑	❑	❑
Specify other:			

39. **In-feed tylosin**

Have you used in-feed tylosin (trade names Tylan, Tyleco) in the past 12 months?

- ❍ yes
- ❍ no
- ❍ don't know
- ❍ unanswered

SKIP LOGIC HERE – IF RESPONDENT ANSWERED YES THEY PROCEED TO NEXT QUESTION. IF RESPODENT ANSWERED 'NO', 'DON'T KNOW' OR 'UNANSWERED'

THEY SKIP TO NEXT BLOCK

What percentage of lots were given in-feed tylosin in the past 12 months?

_____

Select the reason/s in-feed tylosin was used in the past 12 months (select multiple options)

- ❑ mass treatment in response to a disease outbreak (metaphylaxis)
- ❑ timed/ scheduled treatment of lots (prophylaxis)
- ❑ prevention
- ❑ growth promotion
- ❑ don't know
- ❑ unanswered

For the past 12 months, select the disease syndromes in-feed tylosin was for –

(1) mass treatment, and/or
(2) times/ scheduled treatment, and/or
(3) prevention

	mass treatment	timed/ scheduled treatment	prevention
Respiratory (e.g. laryngitis, pneumonia)	❑	❑	❑
Gut (e.g. acidosis, blood scours, scours, rumen health)	❑	❑	❑
Musculoskeletal (lameness, footrot, swollen joints/ legs, foot abscess, lumpy jaw, wooden tongue)	❑	❑	❑
neurological (e.g. PEM/TEME, dropped ear, circling, pink eye)	❑	❑	❑
Urogenital (e.g. prolapsed prepuce, calving, waterbelly, castration)	❑	❑	❑
Other (e.g. tick fever, honker)	❑	❑	❑
Specify other:			

40. **Tilmicosin**

Have you used tilmicosin by injection (trade names Micotil, Tilmax) in the past 12 months?

- ❍ yes
- ❍ no
- ❍ don't know

❍ unanswered

SKIP LOGIC HERE – IF RESPONDENT ANSWERED 'YES' THEY PROCEED TO NEXT QUESTION. IF RESPODENT ANSWERED 'NO', 'DON'T KNOW' OR 'UNANSWERED' THEY SKIP TO NEXT BLOCK

What percentage of 'pulled' animals were given injectable tilmicosin in the past 12 months?

_____

Nominate the purpose/s of using injectable tilmicosin (individual treatment, mass treatment response, timed or scheduled treatment) and the disease syndromes treated

	Individual	mass treatment	timed/ scheduled treatment
Respiratory (e.g. laryngitis, pneumonia)	❑	❑	❑
Gut (e.g. acidosis, blood scours, scours, rumen health)	❑	❑	❑
Musculoskeletal (lameness, footrot, swollen joints/ legs, foot abscess, lumpy jaw, wooden tongue)	❑	❑	❑
neurological (e.g. PEM/TEME, dropped ear, circling, pink eye)	❑	❑	❑
Urogenital (e.g. prolapsed prepuce, calving, waterbelly, castration)	❑	❑	❑
Other (e.g. tick fever, honker)	❑	❑	❑
Specify other:			

41. **In-feed tilmicosin**

Have you used in-feed tilmicosin (trade names Micotil, Tilmax) in the past 12 months?

❍ yes
❍ no
❍ don't know
❍ unanswered

SKIP LOGIC HERE – IF RESPONDENT ANSWERED 'YES' THEY PROCEED TO NEXT QUESTION. IF RESPODENT ANSWERED 'NO', 'DON'T KNOW' OR 'UNANSWERED' THEY SKIP TO NEXT BLOCK

What percentage of lots were given in-feed tilmicosin in the past 12 months?

Select the reason/s in-feed tilmicosin was used in the past 12 months (select multiple options)

- ❑ mass treatment in response to a disease outbreak (metaphylaxis)
- ❑ timed/ scheduled treatment of lots (prophylaxis)
- ❑ prevention
- ❑ growth promotion
- ❑ don't know
- ❑ unanswered

For the past 12 months, select the disease syndromes in-feed tilmicosin was used for –

(1) mass treatment, and/or
(2) times/ scheduled treatment, and/or
(3) prevention

	mass treatment	timed/ scheduled treatment	prevention
Respiratory (e.g. laryngitis, pneumonia)	❑	❑	❑
Gut (e.g. acidosis, blood scours, scours, rumen health)	❑	❑	❑
Musculoskeletal (lameness, footrot, swollen joints/ legs, foot abscess, lumpy jaw, wooden tongue)	❑	❑	❑
neurological (e.g. PEM/TEME, dropped ear, circling, pink eye)	❑	❑	❑
Urogenital (e.g. prolapsed prepuce, calving, waterbelly, castration)	❑	❑	❑
Other (e.g. tick fever, honker)	❑	❑	❑
Specify other:			

42. Injectable erythromycin

Have you used erythromycin by injection (trade names Erymicin, Gallimycin) in the past 12 months?

- ❍ yes
- ❍ no
- ❍ don't know
- ❍ unanswered

SKIP LOGIC HERE – IF RESPONDENT ANSWERED 'YES' THEY PROCEED TO NEXT QUESTION. IF RESPODENT ANSWERED 'NO', 'DON'T KNOW' OR 'UNANSWERED' THEY SKIP TO NEXT BLOCK

What percentage of 'pulled' animals were given injectable erythromycin in the past 12 months?

_____

Nominate the purpose/s of using injectable erythromycin (individual treatment, mass treatment response, timed or scheduled treatment) and the disease syndromes treated

	Individual	mass treatment	timed/ scheduled treatment
Respiratory (e.g. laryngitis, pneumonia)	❑	❑	❑
Gut (e.g. acidosis, blood scours, scours, rumen health)	❑	❑	❑
Musculoskeletal (lameness, footrot, swollen joints/ legs, foot abscess, lumpy jaw, wooden tongue)	❑	❑	❑
neurological (e.g. PEM/TEME, dropped ear, circling, pink eye)	❑	❑	❑
Urogenital (e.g. prolapsed prepuce, calving, waterbelly, castration)	❑	❑	❑
Other (e.g. tick fever, honker)	❑	❑	❑
Specify other:			

43. Tulathromycin

Have you used tulathromycin by injection (trade name Draxxin) in the past 12 months?

- ❍ yes
- ❍ no
- ❍ don't know
- ❍ unanswered

SKIP LOGIC HERE – IF RESPONDENT ANSWERED 'YES' THEY PROCEED TO NEXT QUESTION. IF RESPODENT ANSWERED 'NO', 'DON'T KNOW' OR 'UNANSWERED' THEY SKIP TO NEXT BLOCK

What percentage of 'pulled' animals were given injectable tulathromycin in the past 12 months?

_____

Nominate the purpose/s of using injectable tulathromycin (individual treatment, mass treatment response, timed or scheduled treatment) and the disease syndromes treated

	Individual	mass treatment	timed/ scheduled treatment
Respiratory (e.g. laryngitis, pneumonia)	❑	❑	❑
Gut (e.g. acidosis, blood scours, scours, rumen health)	❑	❑	❑
Musculoskeletal (lameness, footrot, swollen joints/ legs, foot abscess, lumpy jaw, wooden tongue)	❑	❑	❑
neurological (e.g. PEM/TEME, dropped ear, circling, pink eye)	❑	❑	❑
Urogenital (e.g. prolapsed prepuce, calving, waterbelly, castration)	❑	❑	❑
Other (e.g. tick fever, honker)	❑	❑	❑
Specify other:			

44. Short-acting oxytetracycline

Have you used short-acting oxytetracycline by injection (trade names Alamycin, Engemycin, Terramycin 100, Tetravet 10) in the past 12 months?

- ❍ yes
- ❍ no
- ❍ don't know
- ❍ unanswered

SKIP LOGIC HERE – IF RESPONDENT ANSWERED 'YES' THEY PROCEED TO NEXT QUESTION. IF RESPODENT ANSWERED 'NO', 'DON'T KNOW' OR 'UNANSWERED' THEY SKIP TO NEXT BLOCK

What percentage of 'pulled' animals were given injectable short-acting oxytetracycline in the past 12 months?

_____

Nominate the purpose/s of using injectable short-acting oxytetracycline (individual treatment, mass treatment response, timed or scheduled treatment) and the disease syndromes treated

	Individual	mass treatment	timed/ scheduled treatment
Respiratory (e.g. laryngitis, pneumonia)	❑	❑	❑
Gut (e.g. acidosis, blood scours, scours, rumen health)	❑	❑	❑
Musculoskeletal (lameness, footrot, swollen joints/ legs, foot abscess, lumpy jaw, wooden tongue)	❑	❑	❑
neurological (e.g. PEM/TEME, dropped ear, circling, pink eye)	❑	❑	❑
Urogenital (e.g. prolapsed prepuce, calving, waterbelly, castration)	❑	❑	❑
Other (e.g. tick fever, honker)	❑	❑	❑
Specify other:			

45. Long-acting oxytetracycline

Have you used long-acting oxytetracycline by injection (trade names Alamycin LA, Bicatop LA, Hexazol LA, Oxytet 200 LA, Terramycin/LA, Tetravet 200 LA) in the past 12 months?

- ❍ yes
- ❍ no
- ❍ don't know
- ❍ unanswered

SKIP LOGIC HERE – IF RESPONDENT ANSWERED 'YES' THEY PROCEED TO NEXT QUESTION. IF RESPODENT ANSWERED 'NO', 'DON'T KNOW' OR 'UNANSWERED' THEY SKIP TO NEXT BLOCK

What percentage of 'pulled' animals were given injectable long-acting oxytetracycline in the past 12 months?

_____

Nominate the purpose/s of using injectable long-acting oxytetracycline (individual treatment, mass treatment response, timed or scheduled treatment) and the disease syndromes treated

	Individual	mass treatment	timed/ scheduled treatment

Respiratory (e.g. laryngitis, pneumonia)	❑	❑	❑
Gut (e.g. acidosis, blood scours, scours, rumen health)	❑	❑	❑
Musculoskeletal (lameness, footrot, swollen joints/ legs, foot abscess, lumpy jaw, wooden tongue)	❑	❑	❑
neurological (e.g. PEM/TEME, dropped ear, circling, pink eye)	❑	❑	❑
Urogenital (e.g. prolapsed prepuce, calving, waterbelly, castration)	❑	❑	❑
Other (e.g. tick fever, honker)	❑	❑	❑
Specify other:			

46. In-feed oxytetracycline or chlortetracycline

Have you used in-feed oxytetracycline or chlortetracycline (trade names CTC200, Oxy-Eco 100, Tetravet 980, Terramycin 200, Terramycin 880) in the past 12 months?

- ❍ yes
- ❍ no
- ❍ don't know
- ❍ unanswered

SKIP LOGIC HERE – IF RESPONDENT ANSWERED 'YES' THEY PROCEED TO NEXT QUESTION. IF RESPODENT ANSWERED 'NO', 'DON'T KNOW' OR 'UNANSWERED' THEY SKIP TO NEXT BLOCK

What percentage of lots were given in-feed oxytetracycline or chlortetracycline in the past 12 months?

_____

Select the reason/s in-feed oxytetracycline or chlortetracycline was used in the past 12 months (select multiple options)

- ❑ mass treatment in response to a disease outbreak (metaphylaxis)
- ❑ timed/ scheduled treatment of lots (prophylaxis)
- ❑ prevention
- ❑ growth promotion
- ❑ don't know
- ❑ unanswered

For the past 12 months, select the disease syndromes in-feed oxytetracycline or chlortetracycline was used for – (1) mass treatment and/or
(2) times/ scheduled treatment, and/or
(3) prevention

	mass treatment	timed/ scheduled treatment	prevention
Respiratory (e.g. laryngitis, pneumonia)	❑	❑	❑
Gut (e.g. acidosis, blood scours, scours, rumen health)	❑	❑	❑
Musculoskeletal (lameness, footrot, swollen joints/ legs, foot abscess, lumpy jaw, wooden tongue)	❑	❑	❑
neurological (e.g. PEM/TEME, dropped ear, circling, pink eye)	❑	❑	❑
Urogenital (e.g. prolapsed prepuce, calving, waterbelly, castration)	❑	❑	❑
Other (e.g. tick fever, honker)	❑	❑	❑
Specify other:			

47. Short-acting ceftiofur

Have you used short-acting ceftiofur by injection (trade names Calefur, Excenel, Norocef) in the past 12 months?

❍ yes
❍ no
❍ don't know
❍ unanswered

SKIP LOGIC HERE – IF RESPONDENT ANSWERED 'YES' THEY PROCEED TO NEXT QUESTION. IF RESPODENT ANSWERED 'NO', 'DON'T KNOW' OR 'UNANSWERED' THEY SKIP TO NEXT BLOCK

What percentage of 'pulled' animals were given injectable short-acting ceftiofur in the past 12 months?

_____

Nominate the purpose/s of using injectable short-acting ceftiofur (individual treatment, mass treatment response, timed or scheduled treatment) and the disease syndromes treated

	Individual	mass treatment	timed/ scheduled treatment
Respiratory (e.g. laryngitis, pneumonia)	❑	❑	❑
Gut (e.g. acidosis, blood scours, scours, rumen health)	❑	❑	❑
Musculoskeletal (lameness, footrot, swollen joints/ legs, foot abscess, lumpy jaw, wooden tongue)	❑	❑	❑
neurological (e.g. PEM/TEME, dropped ear, circling, pink eye)	❑	❑	❑
Urogenital (e.g. prolapsed prepuce, calving, waterbelly, castration)	❑	❑	❑
Other (e.g. tick fever, honker)	❑	❑	❑
Specify other:			

48. **Long-acting ceftiofur**

Have you used long-acting ceftiofur by injection (trade names Excede) in the past 12 months?

- ❍ yes
- ❍ no
- ❍ don't know
- ❍ unanswered

SKIP LOGIC HERE – IF RESPONDENT ANSWERED 'YES' THEY PROCEED TO NEXT QUESTION. IF RESPODENT ANSWERED 'NO', 'DON'T KNOW' OR 'UNANSWERED' THEY SKIP TO NEXT BLOCK

What percentage of 'pulled' animals were given injectable long-acting ceftiofur in the past 12 months?

_____

Nominate the purpose/s of using injectable long-acting ceftiofur (individual treatment, mass treatment response, timed or scheduled treatment) and the disease syndromes treated

	Individual	mass treatment	timed/ scheduled treatment

Respiratory (e.g. laryngitis, pneumonia)	❑	❑	❑
Gut (e.g. acidosis, blood scours, scours, rumen health)	❑	❑	❑
Musculoskeletal (lameness, footrot, swollen joints/ legs, foot abscess, lumpy jaw, wooden tongue)	❑	❑	❑
neurological (e.g. PEM/TEME, dropped ear, circling, pink eye)	❑	❑	❑
Urogenital (e.g. prolapsed prepuce, calving, waterbelly, castration)	❑	❑	❑
Other (e.g. tick fever, honker)	❑	❑	❑
Specify other:			

49. **Florfenicol**

Have you used florfenicol by injection (trade names Nuflor) in the past 12 months?

- ❍ yes
- ❍ no
- ❍ don't know
- ❍ unanswered

SKIP LOGIC HERE – IF RESPONDENT ANSWERED 'YES' THEY PROCEED TO NEXT QUESTION. IF RESPODENT ANSWERED 'NO', 'DON'T KNOW' OR 'UNANSWERED' THEY SKIP TO NEXT BLOCK

What percentage of 'pulled' animals were given injectable florfenicol in the past 12 months?

_____

Nominate the purpose/s of using injectable florfenicol (individual treatment, mass treatment response, timed or scheduled treatment) and the disease syndromes treated

	Individual	mass treatment	timed/ scheduled treatment
Respiratory (e.g. laryngitis, pneumonia)	❑	❑	❑

Gut (e.g. acidosis, blood scours, scours, rumen health)	❑	❑	❑
Musculoskeletal (lameness, footrot, swollen joints/ legs, foot abscess, lumpy jaw, wooden tongue)	❑	❑	❑
neurological (e.g. PEM/TEME, dropped ear, circling, pink eye)	❑	❑	❑
Urogenital (e.g. prolapsed prepuce, calving, waterbelly, castration)	❑	❑	❑
Other (e.g. tick fever, honker)	❑	❑	❑
Specify other:			

50. Short-acting penicillin

Have you used short-acting penicillin by injection (trade names Depocillin, Norocillin SA, Penethaject, Propercillin) in the past 12 months?

- ❍ yes
- ❍ no
- ❍ don't know
- ❍ unanswered

SKIP LOGIC HERE – IF RESPONDENT ANSWERED 'YES' THEY PROCEED TO NEXT QUESTION. IF RESPODENT ANSWERED 'NO', 'DON'T KNOW' OR 'UNANSWERED' THEY SKIP TO NEXT BLOCK

What percentage of 'pulled' animals were given injectable short-acting penicillin in the past 12 months?

_____

Nominate the purpose/s of using injectable short-acting penicillin (individual treatment, mass treatment response, timed or scheduled treatment) and the disease syndromes treated

	Individual	mass treatment	timed/ scheduled treatment
Respiratory (e.g. laryngitis, pneumonia)	❑	❑	❑

Gut (e.g. acidosis, blood scours, scours, rumen health)	❑	❑	❑
Musculoskeletal (lameness, footrot, swollen joints/ legs, foot abscess, lumpy jaw, wooden tongue)	❑	❑	❑
neurological (e.g. PEM/TEME, dropped ear, circling, pink eye)	❑	❑	❑
Urogenital (e.g. prolapsed prepuce, calving, waterbelly, castration)	❑	❑	❑
Other (e.g. tick fever, honker)	❑	❑	❑
Specify other:			

51. Long-acting penicillin

Have you used long-acting penicillin by injection (trade names Benacillin, Norocillin LA, Ultrapen LA) in the past 12 months?

- ❍ yes
- ❍ no
- ❍ don't know
- ❍ unanswered

SKIP LOGIC HERE – IF RESPONDENT ANSWERED 'YES' THEY PROCEED TO NEXT QUESTION. IF RESPODENT ANSWERED 'NO', 'DON'T KNOW' OR 'UNANSWERED' THEY SKIP TO NEXT BLOCK

What percentage of 'pulled' animals were given injectable long-acting penicillin in the past 12 months?

_____

Nominate the purpose/s of using long-acting penicillin (individual treatment, mass treatment response, timed or scheduled treatment) and the disease syndromes treated

	Individual	mass treatment	timed/ scheduled treatment
Respiratory (e.g. laryngitis, pneumonia)	❑	❑	❑

Gut (e.g. acidosis, blood scours, scours, rumen health)	❑	❑	❑
Musculoskeletal (lameness, footrot, swollen joints/ legs, foot abscess, lumpy jaw, wooden tongue)	❑	❑	❑
neurological (e.g. PEM/TEME, dropped ear, circling, pink eye)	❑	❑	❑
Urogenital (e.g. prolapsed prepuce, calving, waterbelly, castration)	❑	❑	❑
Other (e.g. tick fever, honker)	❑	❑	❑
Specify other:			

52. **Amoxicillin**

Have you used amoxicillin by injection (trade names Betamox, Bimoxyl, Bomox, Moxylan) in the past 12 months?

- ❍ yes
- ❍ no
- ❍ don't know
- ❍ unanswered

SKIP LOGIC HERE – IF RESPONDENT ANSWERED 'YES' THEY PROCEED TO NEXT QUESTION. IF RESPODENT ANSWERED 'NO', 'DON'T KNOW' OR 'UNANSWERED' THEY SKIP TO NEXT BLOCK

What percentage of 'pulled' animals were given injectable amoxicillin in the past 12 months?

_____

Nominate the purpose/s of using injectable amoxicillin (individual treatment, mass treatment response, timed or scheduled treatment) and the disease syndromes treated

	Individual	mass treatment	timed/ scheduled treatment
Respiratory (e.g. laryngitis, pneumonia)	❑	❑	❑
Gut (e.g. acidosis, blood scours, scours, rumen health)	❑	❑	❑

Musculoskeletal (lameness, footrot, swollen joints/ legs, foot abscess, lumpy jaw, wooden tongue)	❑	❑	❑
neurological (e.g. PEM/TEME, dropped ear, circling, pink eye)	❑	❑	❑
Urogenital (e.g. prolapsed prepuce, calving, waterbelly, castration)	❑	❑	❑
Other (e.g. tick fever, honker)	❑	❑	❑
Specify other:			

53. Trimethoprim and/or sulphonamides

Have you used trimethoprim and/or sulphonamides by injection (trade names Amphoprim, SD333 Sulfadimidine, TMPS 240, Tribactral, Triprim, Trisoprim 480, Trivetrin) in the past 12 months?

- ❍ yes
- ❍ no
- ❍ don't know
- ❍ unanswered

SKIP LOGIC HERE – IF RESPONDENT ANSWERED 'YES' THEY PROCEED TO NEXT QUESTION. IF RESPODENT ANSWERED 'NO', 'DON'T KNOW' OR 'UNANSWERED' THEY SKIP TO NEXT BLOCK

What percentage of 'pulled' animals were given injectable trimethoprim/ sulphonamides in the past 12 months?

_____

Nominate the purpose/s of using injectable trimethoprim/ sulphonamides (individual treatment, mass treatment response, timed or scheduled treatment) and the disease syndromes treated

	Individual	mass treatment	timed/ scheduled treatment
Respiratory (e.g. laryngitis, pneumonia)	❑	❑	❑
Gut (e.g. acidosis, blood scours, scours, rumen health)	❑	❑	❑

Musculoskeletal (lameness, footrot, swollen joints/ legs, foot abscess, lumpy jaw, wooden tongue)	❑	❑	❑
neurological (e.g. PEM/TEME, dropped ear, circling, pink eye)	❑	❑	❑
Urogenital (e.g. prolapsed prepuce, calving, waterbelly, castration)	❑	❑	❑
Other (e.g. tick fever, honker)	❑	❑	❑
Specify other:			

54. In-feed trimethoprim and/or sulphonamides

Have you used in-feed trimethoprim and/or sulphonamides (trade names Sulphatrim, Sulprim, Trimidine) in the past 12 months?

- ❍ yes
- ❍ no
- ❍ don't know
- ❍ unanswered

SKIP LOGIC HERE – IF RESPONDENT ANSWERED 'YES' THEY PROCEED TO NEXT QUESTION. IF RESPODENT ANSWERED 'NO', 'DON'T KNOW' OR 'UNANSWERED' THEY SKIP TO NEXT BLOCK

What percentage of lots were given in-feed trimethoprim/ sulphonamides in the past 12 months?

_____

Select the reason/s in-feed trimethoprim/ sulphonamides were used in the past 12 months (select multiple options)

- ❑ mass treatment in response to a disease outbreak (metaphylaxis)
- ❑ timed/ scheduled treatment of lots (prophylaxis)
- ❑ prevention
- ❑ growth promotion
- ❑ don't know
- ❑ unanswered

For the past 12 months, select the disease syndromes in-feed trimethoprim/ sulphonamides was used for –

(1) mass treatment, and/or
(2) times/ scheduled treatment, and/or
(3) prevention

	mass treatment	timed/ scheduled treatment	prevention
Respiratory (e.g. laryngitis, pneumonia)	❑	❑	❑
Gut (e.g. acidosis, blood scours, scours, rumen health)	❑	❑	❑
Musculoskeletal (lameness, footrot, swollen joints/ legs, foot abscess, lumpy jaw, wooden tongue)	❑	❑	❑
neurological (e.g. PEM/TEME, dropped ear, circling, pink eye)	❑	❑	❑
Urogenital (e.g. prolapsed prepuce, calving, waterbelly, castration)	❑	❑	❑
Other (e.g. tick fever, honker)	❑	❑	❑
Specify other:			

55. **Neomycin**

Have you used neomycin by injection (trade names Neomycin-penicillin, neomycin sulphate) in the past 12 months?

❍ yes
❍ no
❍ don't know
❍ unanswered

SKIP LOGIC HERE – IF RESPONDENT ANSWERED 'YES' THEY PROCEED TO NEXT QUESTION. IF RESPODENT ANSWERED 'NO', 'DON'T KNOW' OR 'UNANSWERED' THEY SKIP TO NEXT BLOCK

What percentage of 'pulled' animals were given injectable neomycin in the past 12 months?

_____

Nominate the purpose/s of using injectable neomycin (individual treatment, mass treatment response, timed or scheduled treatment) and the disease syndromes treated

	Individual	mass treatment	timed/ scheduled treatment

Respiratory (e.g. laryngitis, pneumonia)	❑	❑	❑
Gut (e.g. acidosis, blood scours, scours, rumen health)	❑	❑	❑
Musculoskeletal (lameness, footrot, swollen joints/ legs, foot abscess, lumpy jaw, wooden tongue)	❑	❑	❑
neurological (e.g. PEM/TEME, dropped ear, circling, pink eye)	❑	❑	❑
Urogenital (e.g. prolapsed prepuce, calving, waterbelly, castration)	❑	❑	❑
Other (e.g. tick fever, honker)	❑	❑	❑
Specify other:			

56. **Gentamicin**

Have you used gentamicin by injection (trade names Gentam, Gentamax) in the past 12 months?

- ❍ yes
- ❍ no
- ❍ don't know
- ❍ unanswered

SKIP LOGIC HERE – IF RESPONDENT ANSWERED 'YES' THEY PROCEED TO NEXT QUESTION. IF RESPODENT ANSWERED 'NO', 'DON'T KNOW' OR 'UNANSWERED' THEY SKIP TO NEXT BLOCK

What percentage of 'pulled' animals were given injectable gentamicin in the past 12 months?

_____

Nominate the purpose/s of using injectable gentamicin (individual treatment, mass treatment response, timed or scheduled treatment) and the disease syndromes treated

	Individual	mass treatment	timed/ scheduled treatment
Respiratory (e.g. laryngitis, pneumonia)	❑	❑	❑

Gut (e.g. acidosis, blood scours, scours, rumen health)	❑	❑	❑
Musculoskeletal (lameness, footrot, swollen joints/ legs, foot abscess, lumpy jaw, wooden tongue)	❑	❑	❑
neurological (e.g. PEM/TEME, dropped ear, circling, pink eye)	❑	❑	❑
Urogenital (e.g. prolapsed prepuce, calving, waterbelly, castration)	❑	❑	❑
Other (e.g. tick fever, honker)	❑	❑	❑
Specify other:			

57. **Enrofloxacin**

Have you used enrofloxacin by injection (trade names Baytril, Enrotril) in the past 12 months?

- ❍ yes
- ❍ no
- ❍ don't know
- ❍ unanswered

SKIP LOGIC HERE – IF RESPONDENT ANSWERED 'YES' THEY PROCEED TO NEXT QUESTION. IF RESPODENT ANSWERED 'NO', 'DON'T KNOW' OR 'UNANSWERED' THEY SKIP TO NEXT BLOCK

What percentage of 'pulled' animals were given injectable enrofloxacin in the past 12 months?

_____

Nominate the purpose/s of using injectable enrofloxacin (individual treatment, mass treatment response, timed or scheduled treatment) and the disease syndromes treated

	Individual	mass treatment	timed/ scheduled treatment
Respiratory (e.g. laryngitis, pneumonia)	❑	❑	❑
Gut (e.g. acidosis, blood scours, scours, rumen health)	❑	❑	❑

Musculoskeletal (lameness, footrot, swollen joints/ legs, foot abscess, lumpy jaw, wooden tongue)	❑	❑	❑
neurological (e.g. PEM/TEME, dropped ear, circling, pink eye)	❑	❑	❑
Urogenital (e.g. prolapsed prepuce, calving, waterbelly, castration)	❑	❑	❑
Other (e.g. tick fever, honker)	❑	❑	❑
Specify other:			

58. **In-feed virginiamycin**

Have you used in-feed virginiamycin (trade name Eskalin) in the past 12 months?

- ❍ yes
- ❍ no
- ❍ don't know
- ❍ unanswered

SKIP LOGIC HERE – IF RESPONDENT ANSWERED 'YES' THEY PROCEED TO NEXT QUESTION. IF RESPODENT ANSWERED 'NO', 'DON'T KNOW' OR 'UNANSWERED' THEY SKIP TO NEXT BLOCK

What percentage of lots were given in-feed virginiamycin in the past 12 months?

_____

Select the reason/s in-feed virginiamycin was used in the past 12 months (select multiple options)

- ❑ mass treatment in response to a disease outbreak (metaphylaxis)
- ❑ timed/ scheduled treatment of lots (prophylaxis)
- ❑ prevention
- ❑ growth promotion
- ❑ don't know
- ❑ unanswered

For the past 12 months, select the disease syndromes in-feed virginiamycin was used for –

(1) mass treatment, and/or
(2) times/ scheduled treatment, and/or
(3) prevention

	mass treatment	timed/ scheduled treatment	prevention
Respiratory (e.g. laryngitis, pneumonia)	❑	❑	❑
Gut (e.g. acidosis, blood scours, scours, rumen health)	❑	❑	❑
Musculoskeletal (lameness, footrot, swollen joints/ legs, foot abscess, lumpy jaw, wooden tongue)	❑	❑	❑
neurological (e.g. PEM/TEME, dropped ear, circling, pink eye)	❑	❑	❑
Urogenital (e.g. prolapsed prepuce, calving, waterbelly, castration)	❑	❑	❑
Other (e.g. tick fever, honker)	❑	❑	❑
Specify other:			

59. **In-feed monensin**

Have you used in-feed monensin (trade name Elancoban, Moneco, PhibroMonensin, Rumensin) in the past 12 months?

- ❍ yes
- ❍ no
- ❍ don't know
- ❍ unanswered

SKIP LOGIC HERE – IF RESPONDENT ANSWERED 'YES' THEY PROCEED TO NEXT QUESTION. IF RESPODENT ANSWERED 'NO', 'DON'T KNOW' OR 'UNANSWERED' THEY SKIP TO NEXT BLOCK

What percentage of lots were given in-feed monensin in the past 12 months?

_____

Select the reason/s in-feed monensin was used in the past 12 months (select multiple options)

- ❑ mass treatment in response to a disease outbreak (metaphylaxis)
- ❑ timed/ scheduled treatment of lots (prophylaxis)
- ❑ prevention
- ❑ growth promotion
- ❑ don't know
- ❑ unanswered

For the past 12 months, select the disease syndromes in-feed monensin was used for –

(1) mass treatment, and/or
(2) times/ scheduled treatment, and/or
(3) prevention

	mass treatment	timed/ scheduled treatment	prevention
Respiratory (e.g. laryngitis, pneumonia)	❑	❑	❑
Gut (e.g. acidosis, blood scours, scours, rumen health)	❑	❑	❑
Musculoskeletal (lameness, footrot, swollen joints/ legs, foot abscess, lumpy jaw, wooden tongue)	❑	❑	❑
neurological (e.g. PEM/TEME, dropped ear, circling, pink eye)	❑	❑	❑
Urogenital (e.g. prolapsed prepuce, calving, waterbelly, castration)	❑	❑	❑
Other (e.g. tick fever, honker)	❑	❑	❑
Specify other:			

60. **In-feed salinomycin**

Have you used in-feed salinomycin (trade name Posistac, Saleco) in the past 12 months?

❍ yes
❍ no
❍ don't know
❍ unanswered

SKIP LOGIC HERE – IF RESPONDENT ANSWERED 'YES' THEY PROCEED TO NEXT QUESTION. IF RESPODENT ANSWERED 'NO', 'DON'T KNOW' OR 'UNANSWERED' THEY SKIP TO NEXT BLOCK

What percentage of lots were given in-feed salinomycin in the past 12 months?

_____

Select the reason/s in-feed salinomycin was used in the past 12 months (select multiple options)

❑ mass treatment in response to a disease outbreak (metaphylaxis)
❑ timed/ scheduled treatment of lots (prophylaxis)

- ❑ prevention
- ❑ growth promotion
- ❑ don't know
- ❑ unanswered

For the past 12 months, select the disease syndromes in-feed salinomycin was used for –

(1) mass treatment, and/or
(2) times/ scheduled treatment, and/or
(3) prevention

	mass treatment	timed/ scheduled treatment	prevention
Respiratory (e.g. laryngitis, pneumonia)	❑	❑	❑
Gut (e.g. acidosis, blood scours, scours, rumen health)	❑	❑	❑
Musculoskeletal (lameness, footrot, swollen joints/ legs, foot abscess, lumpy jaw, wooden tongue)	❑	❑	❑
neurological (e.g. PEM/TEME, dropped ear, circling, pink eye)	❑	❑	❑
Urogenital (e.g. prolapsed prepuce, calving, waterbelly, castration)	❑	❑	❑
Other (e.g. tick fever, honker)	❑	❑	❑
Specify other:			

61. **In-feed lasalocid**

Have you used in-feed lasalocid (trade name Bovatec) in the past 12 months?

- ❍ yes
- ❍ no
- ❍ don't know
- ❍ unanswered

SKIP LOGIC HERE – IF RESPONDENT ANSWERED 'YES' THEY PROCEED TO NEXT QUESTION. IF RESPODENT ANSWERED 'NO', 'DON'T KNOW' OR 'UNANSWERED' THEY SKIP TO NEXT BLOCK

What percentage of lots were given in-feed lasalocid in the past 12 months?

Select the reason/s in-feed lasolacid was used in the past 12 months (select multiple options)

- ❑ mass treatment in response to a disease outbreak (metaphylaxis)
- ❑ timed/ scheduled treatment of lots (prophylaxis)
- ❑ prevention
- ❑ growth promotion
- ❑ don't know
- ❑ unanswered

For the past 12 months, select the disease syndromes in-feed lasolacid was used for –

(1) mass treatment, and/or
(2) times/ scheduled treatment, and/or
(3) prevention

	mass treatment	timed/ scheduled treatment	prevention
Respiratory (e.g. laryngitis, pneumonia)	❑	❑	❑
Gut (e.g. acidosis, blood scours, scours, rumen health)	❑	❑	❑
Musculoskeletal (lameness, footrot, swollen joints/ legs, foot abscess, lumpy jaw, wooden tongue)	❑	❑	❑
neurological (e.g. PEM/TEME, dropped ear, circling, pink eye)	❑	❑	❑
Urogenital (e.g. prolapsed prepuce, calving, waterbelly, castration)	❑	❑	❑
Other (e.g. tick fever, honker)	❑	❑	❑
Specify other:			

62. **In-feed narasin**

Have you used in-feed narasin (trade name Maxiban, Monteban) in the past 12 months?

- ❍ yes
- ❍ no
- ❍ don't know
- ❍ unanswered

SKIP LOGIC HERE – IF RESPONDENT ANSWERED 'YES' THEY PROCEED TO NEXT

QUESTION. IF RESPODENT ANSWERED 'NO', 'DON'T KNOW' OR 'UNANSWERED' THEY SKIP TO NEXT BLOCK

What percentage of lots were given in-feed narasin in the past 12 months?

_____

Select the reason/s in-feed narasin was used in the past 12 months (select multiple options)

- ❑ mass treatment in response to a disease outbreak (metaphylaxis)
- ❑ timed/ scheduled treatment of lots (prophylaxis)
- ❑ prevention
- ❑ growth promotion
- ❑ don't know
- ❑ unanswered

For the past 12 months, select the disease syndromes in-feed narasin was used for –

(1) mass treatment, and/or
(2) times/ scheduled treatment, and/or
(3) prevention

	mass treatment	timed/ scheduled treatment	prevention
Respiratory (e.g. laryngitis, pneumonia)	❑	❑	❑
Gut (e.g. acidosis, blood scours, scours, rumen health)	❑	❑	❑
Musculoskeletal (lameness, footrot, swollen joints/ legs, foot abscess, lumpy jaw, wooden tongue)	❑	❑	❑
neurological (e.g. PEM/TEME, dropped ear, circling, pink eye)	❑	❑	❑
Urogenital (e.g. prolapsed prepuce, calving, waterbelly, castration)	❑	❑	❑
Other (e.g. tick fever, honker)	❑	❑	❑
Specify other:			

63. **In-feed flavophospholipol**

Have you used in-feed flavophospholipol (trade name Flaveco) in the past 12 months?

- ❍ yes
- ❍ no

- ❍ don't know
- ❍ unanswered

SKIP LOGIC HERE – IF RESPONDENT ANSWERED 'YES' THEY PROCEED TO NEXT QUESTION. IF RESPODENT ANSWERED 'NO', 'DON'T KNOW' OR 'UNANSWERED' THEY SKIP TO NEXT BLOCK

What percentage of lots were given in-feed flavophospolipol in the past 12 months?

_____

Select the reason/s in-feed flavophospholipol was used in the past 12 months (select multiple options)

- ❑ mass treatment in response to a disease outbreak (metaphylaxis)
- ❑ timed/ scheduled treatment of lots (prophylaxis)
- ❑ prevention
- ❑ growth promotion
- ❑ don't know
- ❑ unanswered

For the past 12 months, select the disease syndromes in-feed flavophospholipol was used for –

(1) mass treatment, and/or
(2) times/ scheduled treatment, and/or
(3) prevention

	mass treatment	timed/ scheduled treatment	prevention
Respiratory (e.g. laryngitis, pneumonia)	❑	❑	❑
Gut (e.g. acidosis, blood scours, scours, rumen health)	❑	❑	❑
Musculoskeletal (lameness, footrot, swollen joints/ legs, foot abscess, lumpy jaw, wooden tongue)	❑	❑	❑
neurological (e.g. PEM/TEME, dropped ear, circling, pink eye)	❑	❑	❑
Urogenital (e.g. prolapsed prepuce, calving, waterbelly, castration)	❑	❑	❑
Other (e.g. tick fever, honker)	❑	❑	❑
Specify other:			

64. Quality assurance questions

How often does a registered veterinarian visit the feedlot?

- ❍ never
- ❍ once a year
- ❍ twice a year
- ❍ four times a year
- ❍ monthly
- ❍ more than once a month
- ❍ unanswered

Does the feedlot have a protocol such as a 'documented processing protocol' for inducting new animals into the feedlot?

- ❍ yes
- ❍ no
- ❍ don't know
- ❍ unanswered

Has a veterinarian issued the feedlot with a treatment protocol/ schedule or 'prescribed veterinary medicine and veterinary chemical list' in the past 12 months?

- ❍ yes
- ❍ no
- ❍ don't know
- ❍ unanswered

SKIP LOGIC HERE – IF RESPONDENT ANSWERED 'YES' THEY PROCEED TO NEXT QUESTION. IF RESPODENT ANSWERED 'NO', 'DON'T KNOW' OR 'UNANSWERED' THEY SKIP THE NEXT TWO QUESTIONS

Is the treatment protocol/ 'prescribed list' followed by feedlot staff? (select the most appropriate option)

- ❍ always (100% of the time)
- ❍ frequently (approx 80% of the time)
- ❍ often (approx 60% of the time)
- ❍ occasionally (approx 40% of the time)
- ❍ seldom (
- ❍ never
- ❍ unanswered

Are sick animals assessed for their response to treatments before they are returned to their 'home pen'?

- ❍ yes
- ❍ no
- ❍ don't know

- ❍ unanswered

Who supplies veterinary prescription drugs (e.g. S4 chemicals such as antibiotics, antiinflammatories) for use in the feedlot (can select multiple options)

- ❑ consulting vet
- ❑ other vet
- ❑ online
- ❑ don't know
- ❑ unanswered

Where do you buy animal health products such as drenches and pesticides (i.e. over-thecounter products) for use in the feedlot? (can select multiple options)

- ❑ vet
- ❑ online
- ❑ rural merchandise store
- ❑ other _____
- ❑ don't know
- ❑ unanswered

Who has access to veterinary prescription drugs at the feedlot? (can select multiple options)

- ❑ animal health crew/ stock handlers
- ❑ feeding crew/ maintenance crew
- ❑ management
- ❑ Other _____
- ❑ don't know
- ❑ unanswered

Who can access animal health products such as drenches and pesticides at the feedlot? (can select multiple options)

- ❑ animal health crew/ stock handlers
- ❑ feeding crew/ maintenance crew
- ❑ management
- ❑ Other _____
- ❑ don't know
- ❑ unanswered

Who can administer veterinary prescription drugs to animals at the feedlot? (can select multiple options)

- ❑ animal health crew/ stock handlers
- ❑ feeding crew/ maintenance crew
- ❑ management
- ❑ Other _____
- ❑ don't know
- ❑ unanswered

Who administers animal health products (drenches, pesticides) to animals at the feedlot?

(can select multiple options)

- ❑ animal health crew/ stock handlers
- ❑ feeding crew/ maintenance crew
- ❑ management
- ❑ Other _____
- ❑ don't know
- ❑ unanswered

How do you identify animals that have been treated with a prescription drug (with an applicable withholding period)? (select one option)

- ❍ hospital tag only
- ❍ management computer software only
- ❍ hospital tag AND management computer software
- ❍ do not identify treated animals
- ❍ other _____
- ❍ don't know
- ❍ unanswered

What training is provided to staff who administer veterinary prescription drugs (e.g. antibiotics, anti-inflammatories) to animals? (can select multiple options)

- ❑ staff trained by the feedlot veterinarian
- ❑ staff trained by the livestock supervisor
- ❑ off-site courses, seminars such as ChemCert
- ❑ other _____
- ❑ don't know
- ❑ unanswered

Is the main storage area for veterinary prescription drugs locked at all times?

- ❍ yes
- ❍ no
- ❍ don't know
- ❍ unanswered

Are veterinary prescription drugs and animal health products stored according to the label directions e.g. if the drug requires refrigeration is it always kept refrigerated?

- ❍ yes
- ❍ no
- ❍ don't know
- ❍ unanswered

How is veterinary chemical (i.e. prescription and over-the-counter) inventory managed for incoming/ outgoing chemicals? (select one option)

- ❍ computerised records
- ❍ manual entry - record book
- ❍ no records
- ❍ other _____

- ❍ don't know
- ❍ unanswered

How often are veterinary chemical stocks audited? (select one option)

- ❍ never
- ❍ once a year
- ❍ twice a year
- ❍ four times a year
- ❍ monthly
- ❍ more than monthly
- ❍ unanswered

How are out-of-date veterinary chemicals managed? (select one option)

- ❍ immediate disposal
- ❍ vet approved short extension of shelf life
- ❍ used until complete
- ❍ other _____
- ❍ don't know
- ❍ unanswered

Appendix 3. Alternatives to antibiotics for the prevention and treatment of commonly occurring feedlot diseases

Nitric Oxide

Nitric oxide (NO) is a molecule produced endogenously in cattle by nitric oxide synthase with production increased in macrophages in response to infectious or inflammatory diseases (MacMicking et al., 1997). Sustained synthesis of nitric oxide confers cytostatic or cytotoxic activity on macrophages against viruses, bacteria, fungi, protozoa, helminths and tumour cells, with enhancement of this effect by other macrophage products including glutathione, cysteine, hydrogen peroxide or superoxide (MacMicking et al., 1997). Being both hydrophilic and lipophilic, nitric oxide readily crosses cell membranes. At low concentrations, nitric oxide promotes the growth and activity of immune cells, whilst at high concentrations it reacts with oxygen or superoxide to produce reactive nitrogen and oxygen intermediates with antimicrobial properties effected by DNA damage, enzyme inhibition and lipid peroxidation (Regev-Shoshani et al., 2014). Thus, nitric oxide metabolites are both antimicrobial and host animal cytotoxic. The rationale underlying the use of nitric oxide as a bovine respiratory disease (BRD) preventative or treatment, is the greater ability of eukaryotic cells to survive oxidative and nitrosative stress due to higher concentrations of antioxidants such as glutathione peroxidase, compared with prokaryotic microbes (Miller et al., 2007). This emphasises the importance of establishing an appropriate dose rate to inhibit or kill microbes without injuring the animal tissues exposed to increased concentrations of nitric oxide in excess of endogenous production. Also, antimicrobial effects from nitric oxide might be more safely achieved if the antioxidant status of the animal could be enhanced prior to treatment. In practice, this is possible with the inclusion of elevated concentrations of selenium and vitamin E in backgrounding diets with sufficient fermentable carbohydrate to maintain blood glucose concentrations conducive to hepatic ascorbate synthesis. Conversely, the antioxidant status of cattle placed directly in the feedlot cannot be immediately enhanced.

Regev-Shoshani et al. (2013) sprayed a suspension delivering 160 ppm of nitric oxide, or a saline placebo, into both nostrils of 82 saleyard purchased cattle newly arrived at a Canadian feedlot after 4-6 h of transport. There was a reduction ($P < 0.001$) in BRD incidence in the nitric oxide treated group over the first 14 d, but there was no significant difference between groups over the first 28
d. Peak BRD incidence in the control cattle occurred in the first week, with a delay in peak incidence in the nitric oxide treated cattle to the third week. Mortality data were not presented but a difference with a total sample size of 82 would be highly unlikely. More recently, Regev- Shoshani et al. (2017) showed equivalence in BRD incidence, average daily gain and mortality rate between the nitric oxide treatment described above and conventional antibiotic metaphylaxis with 1080 multiple sourced, commingled beef calves considered low-moderate risk for BRD and observed following placement in a Canadian feedlot and fed for 150 d. However, negative controls were not included in this study, so it is unknown whether either treatment had a significant effect compared with no treatment. In a study of cattle at high-risk of developing BRD, Crepieux et al (2016), nasal instillation of a nitric oxide releasing solution was found inferior to conventional antibiotic treatment with tilmicosin. Recently, Timsit et al (2017) hypothesized that this inferiority was due to differences between treatments with regards to their effects on the nasopharyngeal microbiota. They concluded after study of the nasopharyngeal microbiota by culture and non-culture methods that difference in ability to inhibit

colonization of the nasopharynx by Pasteurellaceae may be the basis for nitric oxide being inferior to tilmicosin for control of BRD in high-risk cattle.

The listed studies suggest that further research into the effects of nitric oxide as an induction treatment for the reduction of BRD incidence under commercial feedlot conditions is warranted, with additional considerations. These considerations should include: negative controls; evaluation of the most appropriate dose rate; and assessment of the potential positive effects of increased tissue concentrations of the antioxidants, vitamin E, glutathione peroxidase, and ascorbate.

Plant Extracts

Several reviews of the potential for plant extracts to have positive effects and livestock health and production have been written, with some reporting statistically weak results as support for the use of these products (Papatsiros et al., 2012). Whilst screening studies of a range of whole plant and plant extracts are essential for the possible identification of biological molecules that might improve health or production in cattle, the problem inherent to this screening approach is that positive outcomes will occur over time purely due to chance. This effect is likely exacerbated by the common use of Latin square study designs (cross –over studies where recent nutritional history can alter the effects of subsequent trial compounds) and small sample sizes (frequently n = 4, eg. Yang et al., 2010; Fandino et al., 2008). This explains some of the variability in outcomes measured in response to feeding plant extracts. Therefore, screening arrays of plant products (eg. Durmic et al., 2014) should only be considered to be a starting point for further investigation of products identified as having potential positive effects from the first round of testing. Initial screening with subsequent adequately replicated targeted research with defined compounds of known concentrations and administration rates is standard practice for conventional pharmaceutical research and development companies. This rigour does not appear to apply to investigation of the potential for plant extracts as replacements for conventional antimicrobials. The apparent ideological desire for “natural” products has prompted some researchers to suggest that the most effective means of delivering plant derived secondary metabolites is to feed whole fresh or dried plant material (Papatsiros et al., 2012). The rationale underlying this is unclear. Absence of characterisation of active ingredients with these products can result in varying responses, and possibly unforeseen negative effects arising from disturbance of the rumen microflora or interactions with other compounds in the rumen thereby interfering with nutrient availability. On this basis, whole plant products, or complex combinations of compounds in plant extracts do not warrant further investigation in commercial applications by the feedlot sector, unless research bodies first fully characterise the active ingredients and establish efficacy and safety with *in vitro* testing.

Some plant extracts have been identified and refined, with subsequent research to measure their effects on the rumen microbiome, fermentation output and ruminant production and health. Of these, condensed tannins have been shown to reduce the incidence of bloat in cattle on pasture (Waghorn and Jones, 1989) and in sheep on lush pasture (Waghorn et al., 2002), in addition to reducing nematode burdens in lambs on a low protein diet (Butter et al., 2000). However, condensed tannins have been shown to precipitate 50% of the soluble protein in the rumen (Waghorn and Jones, 1989), which is presumably the pathway by which pasture bloat incidence is reduced. Further, a primary pathway for feedlot bloat is the overgrowth of *Streptococcus bovis* and the contribution of its

slimy capsule to ingesta vicosity and the direct contribution of lowered pH to ingesta viscosity. Therefore, the binding of soluble protein by condensed tannins is of no value to the prevention of feedlot bloat and could have negative effects on production if it limited microbial protein production.

A major impediment to the potential use of many plant extracts, particularly essential oils, is their generalised antimicrobial effects, leading to a reduction in total volatile fatty acid production (Durmic et al., 2014; Benchaar et al., 2008). This obviously precludes their use as therapeutic agents. Some Australian plant extracts appear not to inhibit total volatile fatty acid production *in vitro*, such as *Eremophila glabra* (Durmic et al., 2014), which has shown similar efficacy in the maintenance of rumen pH, VFA production and lactate suppression, as virginiamycin, in sheep ($P < 0.001$; n = 8) challenged with cracked wheat, at a dose of 400g/kg wheat (Durmic et al., 2012). Further, the plant extract from the Australian plant, *Chameacystisis palmensis* was shown with *in vitro* screening to increase total VFA production (Durmic et al., 2014). It is clearly impractical to dilute the supply of grain in a feedlot diet by the inclusion of a plant extract additive at 40% of the grain by weight, which further emphasises the requirement for identification and refinement of the active ingredients that might assist with the prevention of lactic acidosis or enhance production through more general effects on rumen fermentation efficiency. Thus, extensive laboratory work with these compounds is required before their consideration in controlled randomised feeding studies of larger sample sizes than have commonly been used, and their subsequent evaluation in large, randomised commercial studies. In addition, it will be important to determine if antimicrobial resistance is selected by exposure of bacteria to the selected plants of interest, their extracts or isolated active components.

Supplemental Yeast or Yeast Products

Ponce et al. (2012) found that the addition to the receiving diet of 1.8 g/animal/d of enzymatically hydrolysed yeast extract (Celmanax™) tended ($P = 0.09$) to reduce BRD morbidity. Whilst Finck et al. (2014) found an indirect immunological response to lipopolysaccharide challenge in cattle supplemented with 5 g/animal/d of live yeast (*Saccharomyces cerevisiae* subsp. *boulardii*) or 5 g/animal/d of cell wall from the same yeast species, this did not translate into a reduction ($P = 0.36$) in BRD morbidity. This is consistent with the earlier finding by Keyser et al. (2007), that supplemental yeast (*Saccharomyces cerevisiae* subsp. *boulardii*; Proternative Stress Formula™) fed at a rate of 0.5 g/animal/d, in addition to 1 g/animal of the same product as an oral paste at induction, did not reduce BRD morbidity. Thus, there is a lack of robust evidence to support the use of yeast products to improve health in feedlot cattle, with the aim of reducing antibiotic usage. However, live yeast products have been found to increase productivity in dairy cows (Finck et al., 2014) and in feedlot steers fed a 50% hay/ 50% rolled barley diet (Williams et al., 1991; but note n = 3) through enhanced fibre digestion in the rumen. It therefore follows that positive effects in beef feedlot cattle are more likely in higher fibre diets, and within these, in starter or backgrounding diets with still higher fibre fractions. Additional support for this exists in the form of improvements in dry matter intakes and rumen development in dairy calves for the first 6 wk (Lesmeister et al., 2004), suggesting that the inclusion of *S. cerevisiae* could improve rumen function and feed intake in cattle newly arrived at the feedlot. Further research is therefore warranted with live yeast products with higher fibre feedlot diets during the early feeding period or with backgrounding diets, using larger sample sizes. The effect of mannan oligosaccharides (MOS) from yeast cell walls in binding pathogens such as *E. coli* and *Salmonella* spp., and the effect of this on the gross outcome of morbidity, requires further research based on the

survival of 17 to 34% of yeast cells after transit through the ruminant digestive tract (DurandChaucheyras et al., 1998).

Bacterial Probiotics

Recent studies suggest that integrating probiotics into ruminant feeds might improve various aspects of ruminant performance, mitigate disease, promote overall animal health and well-being, and reduce the environmental impacts of ruminant production. It is believed that probiotics provide these benefits by favourably modulating the microbial environment within the gastrointestinal tract of ruminant animals (Stover et al 2016).

In ruminants, it is logical that lactate utilising bacteria provided in the feed could reduce pH depression with diets high in fermentable carbohydrates. The rumen contains approximately 25 x 10^9 to 50 x 10^9 bacteria. Commercial direct fed microbial products aim to provide bacterial doses in the range of 1 x 10^4 to 1 x 10^9 colony forming units (Galyean et al., 2000) which would appear to be sufficient to have an effect on the composition of the rumen microbial flora assuming a high rate of survival of the bacteria on the feed before ingestion and rumen conditions suitable for proliferation of the species of interest. A research report by Bos Technica Research Services (2009) showed there can be considerable loss of viable bacteria in pouches of *Lactobacillus acidophilus* and *Propionibacterium freudenreichii*, but this could be offset by proliferation of bacteria in delivery lines and in some instances in the mixed feed. However, recovery of bacteria was negligible where the culture was added to hot feed, such as steam flaked grain that had not cooled sufficiently. The effects of hot weather and ration moisture on recovery of viable bacteria have not been adequately determined and might explain some of the variability in results with the use of direct fed bacteria, assuming they have the potential to exert positive effects if delivered at the desired dose.

Another likely contributor to variability in production and health outcomes with direct fed bacteria is the inclusion of lactate producing species. The inclusion of these in ruminants, where we seek to prevent the pH depression inherent to a high rate of VFA production, which can be greatly exacerbated by production of even modest amounts of lactate, is puzzling. It appears that the use of these species in ruminants arose from their use with monogastrics, and the rationale for their use with ruminants is that species such as those from the genera *Lactobacillus* and *Enterococcus* maintain a low and constant rumen concentration of lactate thereby sustaining an active population of lactate utilisers which act to prevent lactate accumulation and marked pH depression (Papatsiros, 2012; Krehbiel et al., 2003). However, the paper cited to support this contention found that maintenance of rumen pH, and digestibility of high moisture ear corn and corn silage, were greatest at the lowest dose of a combination of *Enterococcus faecium*, *Lactobacillus plantarum* and *Saccharomyces cerevisiae* (Nocek et al., 2002). This paper used dairy cows and delivered much higher doses of microbes by rumen catheter than are commonly used in beef cattle, and had no negative controls. However, even in the absence of controls, it is illogical to arrive at a conclusion that these rumen delivered microbes had a positive effect on rumen efficiency and health when the lowest dose corresponded to the greatest improvements.

It has been claimed that probiotics can stimulate the immune system (Krehbiel et al., 2003; Papatsiros, 2012). However, the quoted studies have all been done with monogastrics, including mice (Matsuzaki, 2000) and humans (Chiang et al., 2000). The authors are unaware of any research with ruminants to investigate this claim. Immunomodulatory effects cannot be mediated via the rumen wall, which by necessity presents a substantial barrier to bacteria when healthy and is subjected to multi-species invasion when compromised by inanition (Zhang et al., 2013) or acidosis (Gressley, 2011). However, Brashears et al. (2003) have identified several species of lactobacilli that can survive a pH as low as 2, plus the effects of bile acids up to 0.3%, and these could therefore survive transition through the abomasum into the small intestine, where immunomodulatory effects are theoretically possible. Survival of these organisms has been substantiated through their effects as competitive inhibitors of coliforms in the intestine (Brashears et al., 2003).

Whilst some some research reports provide statistically modest indications that lactate producing bacteria can improve feedlot performance (Galyean et al., 2000), or show no significant effects (Trenkle, 2001), there is a paucity of statistically robust published research papers from peer reviewed journals to support their use or to support the use of direct fed bacteria generally, with some showing no effect on performance (Antunovic et al.,2005; Brashears et al., 2003; Elam et al., 2003) but a significant reduction (P = 0.006; NPC 747 v control) in *E. coli* O157:H7 faecal shedding (Brashears et al., 2003; Elam et al., 2003). An extensive review by Krehbiel et al. (2003) noted the variability in performance results from direct fed bacteria, but examining some published studies and several research reports, found improvements using orthogonal contrasts in ADG. However, the absence of a significant corresponding effect on F:G accompanied by no significant effect on DMI calls this finding into question. It is possible that the variable results with direct fed bacteria have occurred due to the conflicting effects of lactate utilisers and lactate producers in the rumen, particularly considering the lack of logic underlying the use of the study purported to support positive effects from lactate producers in the rumen. However, this confusion does not negate possible positive effects of lactate producers on intestinal health and their potential to exert positive effects on immunity, albeit untested in ruminants. Therefore, it is logical that lactate producers should be fed to establish these in the intestine and lactate utilisers should be fed to maximise their effects on rumen fermentation efficiency and therefore rumen health. On this basis, it would be logical to feed a lactate producer such as *Lactobacillus* spp. early in the feeding period to allow intestinal establishment without ongoing rumen effects, and to feed a lactate utiliser such as *Propionibacterium* spp. for the duration of the feeding period. Support for this contention exists with the results from a phase-feeding study (Huck et al., 2000, in Krehbiel et al., 2003) where *Lactobacillus acidophilus* BG2FO4 and *Propionibacterium freudenreichii* P-63 were fed alone or in sequence with an initial phase of 28 days over a 126 day total feeding period. Feeding either of these bacteria alone through the entire feeding period did not affect daily gain, DMI or feed conversion efficiency. Feeding *P. freudenreichii* for 28 d followed by *L. acidophilus* for the remainder of the feeding period improved ADG but not feed conversion efficiency compared with controls. However, feeding *L. acidophilus* for 28 d followed by *P. freudenreichii* for the remainder of the feeding period resulted in 5% greater ADG and 5.1% greater feed conversion efficiency compared with controls. Further, studies (Greening et al., 1991; Kung and Hession, 1995) have shown that the lactate utiliser *Megasphaera elsdenii* can prevent the accumulation of lactate during the transition from roughage based to high concentrate diets, both *in vitro* and *in vivo*, and *Propionibacterium acidipropionici* has increased propionate yield (Kim et al., 2000).

In summary, the published effects of direct fed bacteria on the performance of feedlot cattle are variable and equivocal. It logically follows that consistent positive effects of direct fed bacteria on the health of feedlot cattle have not been substantiated. Targeted research with these products has suffered from a lack of understanding of their modes of action (noted by Krehbiel et al. in 2003 and remains the case today). To some extent it appears that an erroneous historical attribution of positive rumen fermentation effects from lactate producers has blinded research to their most appropriate application, and, production effects from direct fed bacteria have been confused and possibly negated in some instances by the contrary rumen effects of lactate producers and lactate utilisers. It has been established that lactate producers can reduce faecal shedding of coliforms through competitive inhibition and there is the potential that they might improve intestinal health and systemic immunity. Lactate producers could improve rumen fermentation efficiency through increased propionate yield and could help prevent lactic acidosis on high carbohydrate diets through increased consumption of lactate in the production of propionate. Therefore, further research is warranted to investigate the effects on the health and production of feedlot cattle with the feeding of lactate producers early in the feeding period and lactate utilisers throughout. Clarification of these effects might warrant the use of these products as alternatives to antibiotics to maintain rumen health and to improve production.

While most probiotic preparations are exempt from APVMA registration, products for cattle containing *Bifidobacterium bifidum, Enterococcus faecium, Lactobacillus acidophilus, Lactobacillus delbrueckii* subspecies *bulgaricus, Lactobacillus plantarum, Lactobacillus rhamnosus, Megasphaera elsdenii, Streptococcus salivarius* subspecies *thermophiles* are currently registered.

Organic Acids

Based on *in vitro* fermentation of corn, it has been suggested that organic acids, such as aspartate, fumarate and malate, might provide an alternative to antimicrobial compounds (Callaway and Martin, 1996) to improve the efficiency of rumen fermentation. Malate is an intermediate in the production of succinate or propionate in some ruminal bacteria (Khampa et al., 2006) and malate has stimulated propionate production in *in vitro* cultures (Callaway and Martin, 1996; Martin and Streeter, 1995) and *in vivo* in steers at the low dose of 100 and 200 mg/kg concentrate DM (Kung et al., 1982). Malate has been shown to increase milk production in dairy cows at 70 g/hd/d (Stallcup, 1979, $P < 0.05$) and, ADG and DMI in Holstein bulls at 27.2 g/hd/d (Sanson and Stallcup, 1984). In addition, malate increased N retention in dairy steers on a high carbohydrate diet (Khampa et al.,2006). Malate stimulates some species of ruminal bacteria, such as the lactate utiliser, *Selenomonas ruminantium*, thereby contributing to the maintenance of rumen pH, plus it removes hydrogen ions in its bacterial conversion to propionate (Khampa et al., 2006). Thus, malate shows conderable potential as a non-antimicrobial agent for the improvement of rumen fermentation efficiency and the prevention of lactic acidosis. Further, it might be complementary with lactate utilising direct fed bacteria through the provision of a metabolic intermediate compound to increase their populations and fermentative output.

Bacteriophages

Bacteriophages, commonly referred to as “phages” are viruses that parasitise the genetic machinery of bacteria for replication. Lytic phages then lyse or rupture the bacterial cell to release the next

generation of phages through the production of lysins coded for by the phage and produced by the bacterial intracellular machinery (Knoll and Mylonakis, 2013). Generally, only obligate lytic phages should be used in antibacterial therapy (Oliveira et al., 2015), due to the incorporation of temperate phages into the bacterial genome without killing the host bacterium (lysogeny; Abedon et al., 2011) which can increase the virulence or pathogenicity of the bacterium, or transmit antimicrobial resistance genes (Marti et al., 2014).

Smith et al. (1987) found marked numerical reductions in counts of specific strains of *Escherichia coli* recovered from the intestines or faeces of twin pre-ruminant calves which were dosed orally with corresponding strains of phages (10^5 organisms) isolated on the basis of the K antigen on the bacterium, compared with their negative controls. Statistical analysis of these results was not provided, but the numerical differences were substantial. More recently, Callaway et al. (2008) used phage therapy to significantly reduce the populations of *E. coli* O157:H7 in the faeces, caecum and rectum of sheep, after oral inoculation with the bacterium and the phage at a ratio of 1 colony forming unit: 1 plaque forming unit. The reduction in the *E. coli* O157:H7 concentration in the ingesta and faeces occurred after only 24 h exposure to the phage therapy. This rapid effect is not surprising considering phages multiply exponentially (Knoll and Mylonakis, 2013) and act as a self-amplifying drug (Borie et al., 2014). The converse of the amplification of phages in response to a plentiful supply of the specific host bacterial species is the decline in the phage population in response to the destruction of the host population. Thus, the population of a given phage and its target bacterium vary as in a predator-prey relationship, with initial multiplication of the phage in response to the abundant prey bacterium, and its virtual disappearance as the infection resolves (Harper et al., 2014). It is important to note that the equilibrium that is achieved between the phage and bacterium will vary with different species and the reduction in the bacterial population must be sufficient to prevent clinical disease, sub-clinical disease, or recrudescence, depending on the required outcome for a given infection. There is no generalised relationship in terms of phage and host bacterium population dynamics and the clinical and production effectiveness of the ultimate equilibrium must be established for each phage-bacterium pair. The significance of this interaction has not been adequately recognised previously and emphasise the point that fundamental biological studies must be done to fully describe the behaviour of any phage and its host bacterial species.

There are two major challenges with the use of phages in disease control. The first is the isolation of the appropriate phages for a given production unit because they are highly specific. Smith and Huggins (1982) described a method for phage identification and culture and this must be applied to appropriate samples for a given feedlot. The other challenge to phage disease prevention effectiveness is the propensity for bacteria to produce phage resistant mutants (Smith et al., 1987). This was encountered as a problem in the intensive calf rearing system used as a study site by Smith et al. (1987) and was reported both with short term exposure and with the movement of calves into uncleaned housing. If phage therapy could be utilised with feedlot cattle the contribution of environmental contamination to the development of phage resistance would presumably be minimal with the major bacteria involved in bovine respiratory disease, as these live in the animal, and the survival of organisms in the environment such as *E. coli* is minimal due to desiccation and regular cleaning of water troughs, feed troughs and pens. In addition, it has been proposed that conjugative plasmid dependent phages could selectively target bacterial cells containing conjugative plasmids (Viertel et al., 2014). As many antimicrobial resistant bacteria possess this resistance courtesy of conjugative plasmids encoding AMR genes, their selective killing would suppress horizontal gene

transfer and therefore suppress the proportion of AMR bacteria. Further, as knowledge of the biology of specific phages increases, temperate phages can be engineered to reintroduce genes to make a strain of bacteria susceptible to antibiotics again (Borie et al., 2014). This effect was identified serendipitously in two strains of *E.coli* E12, one a susceptible wild-type and the other an AMR strain with a mutation in its efflux pump that conferred multidrug resistance (Bohnert et al., 2007). Phage based homologous recombination resulted in inactivation of the mutant efflux pump gene and restoration of the wild-type pump with the concomitant return of antibiotic susceptibility. Thus, with enhanced understanding of the biology of phages and their target bacteria, the issue of the development of resistance of bacteria to phages can not only be addressed, but these pathways have the potential, with some bacterial species at least, to reinstate antibiotic susceptibility.

The high specificity of phages is an advantage in terms of preserving beneficial normal flora. Even so called narrow spectrum antibiotics have effects against many species of bacteria, and the removal of desirable bacterial species from a given microbial ecological niche can lead to superinfections of opportunistic undesirable species (Borie et al., 2014). The selective pressure for bacterial mutants resistant to a given phage can be addressed by developing a cocktail of phages directed against a given bacterial species. In this way phage therapy allows a broad spectrum of antibacterial agents to be directed against a single bacterial species, which is the converse of conventional antibiotic therapy.

Phage therapy is superior to the use of antibiotics where it can be targeted against biofilms. Biofilms are aggregations of cells surrounded by a matrix of extracellular polymeric substance (EPS) produced, to at least some extent, by the cells in the biofilm (Harper et al., 2014). Communication between cells in a biofilm can occur through the release of signalling chemical molecules, conferring community activity on the biofilm (Harper et al. 2014). Bacterial biofilms can contain metabolically inactive persister cells which are resistant to antibiotics, but can reactivate after stress (Harper et al., 2014). Thus, bacterial biofilms are resistant to the effects of antibiotics through both the physical effects of preventing access and contact, and, the reactivation of persister cells after the film is stressed by exposure to an antibiotic. Significantly, with respect to bovine respiratory disease (BRD), there is recent *in vitro* evidence that *Mannheimia haemolytica* can form biofilms (Boukahil and Czuprynski, 2015; Haig, 2011). Further, biofilms can be formed by *Pasteurella multocida* (Elswaifi et al., 2012) and *Histophilus somni* (Murray et al., 2016), and these potential BRD pathogens can together form a polymicrobial biofilm (Elswaifi et al., 2012). Clearly, the formation of biofilms could reduce the effectiveness of commonly used antimicrobials in the treatment of BRD, resulting in an elevated BRD case fatality rate. However, many lytic phages can infect and destroy bacteria within biofilms. There are four mechanisms that allow phages to infiltrate and destroy biofilms (Harper et al., 2014):

1. The amplification of bacteriophages by infection of the most accessible bacterial cells then provides phage access to adjoining bacteria deeper within the biofilm. The concomitant reduction in EPS secretion through the destruction of bacteria facilitates progressive removal of the biofilm and suppression of potential regeneration.

2. Phages can directly degrade the EPS through the production of depolymerising enzymes.

3. Phages can induce the production of depolymerising enzymes by the host cell's own genome.

4. The decreased metabolic activity of persister cells does not protect them from infection by phages. Whilst these largely inactive cells do not allow phage replication and dissemination, once the persister cells are reactivated, the intracellular phages can then parasitise the host genetic machinery for replication and lysis.

Methods of Administration: Phage therapy targeting the bacteria involved in BRD could utilise either local treatment with aerosols, or the systemic route with an injectable product (Abedon et al., 2011). The delivery of aerosols is routinely employed in Australian feedlots for the administration of a modified live vaccine against bovine herpesvirus 1 and this equipment would presumably also be suitable for the administration of phages to the respiratory tract. Research is required to determine if phage suspensions targeting BRD agents survive mixing with the vaccine once it is thawed and mixed into its normal saline carrier. The bacterial species most commonly involved in BRD are normal commensal flora of the upper respiratory tract, so an intranasal spray would locate phages selected against *M. haemolytica*, *P. multocida*, and *H. somni* with their hosts. Assuming efficacious lytic phages could be identified and purified against these bacteria, the research question to be addressed is whether, having reached predator-prey equilibria with the bacteria, the phage population would persist for the duration of the feeding period and whether they would co-colonise the lower respiratory tract if these commensal bacteria exploited an opportunity induced by predisposing factors to invade the lungs. With treatment of clinical cases, it is doubtful if sufficient quantities of phage could be introduced to the lungs to overwhelm the invading bacteria. The use of systemic phage administration (through injection) would have to be considered in this instance, which would likely require modification of the selected phage to prevent it's inactivation by the animal's immune system via neutralising antibodies, or removal by the reticuloendothelial system (Oliviera et al., 2015). However, if phage therapy could be effectively employed prophylactically to prevent BRD, the issues of administration to achieve effective treatment obviously become much less significant.

Phages that parasitise bacteria in the gastrointestinal tract survive in faecal pats while their host bacteria survive (Niu et al., 2009; Smith and Huggins, 1982). Earlier work with E. coli phages in calves (Smith and Huggins, 1982) showed stomach tubing with the suspension was an effective administration technique. Therefore, with a phage suspension of sufficient concentration, oral administration with conventional drenching equipment should be possible.

There are opportunities for enhancing the effectiveness of phages for targeted applications. As mentioned above, phages administered systemically might require liposome carriers or engineering to have non-immunogenic and biocompatible surface peptides to avoid inactivation by the immune system (Oliviera, 2015). Also, virulence enhancing factors can be added to the phage genome. Lu and Collins (2007) inserted a gene coding for a glycoside hydrolase with biofilm dispersing activity into *E. coli* bacteriophage T7, thereby increasing its effectiveness in dispersing the biofilm and killing the bacteria within it. Research continues in this approach for the removal of biofilms in industrial processes.

Potential for the Application of Phage Therapy in Feedlot Health Management: There is considerable scope for the use of phage therapy in feedlot health management. However, the following observations drawn from the published literature on phage research must be considered in further research:

- Every phage is different and the relationship it has with its host bacterial species is different. We cannot therefore reliably extrapolate from observations of the effects of one phage strain to another.
- Only lytic phages should be used in routine therapeutic applications. Lysogenic phages should only be used where they have been engineered to reintroduce antibiotic susceptibility to the target bacterial species.
- To prevent rapid development of mutant strains of bacteria with phage resistance, multiple phages directed against a single bacterial species should always be used in combination (a phage cocktail), never alone.
- Phages likely provide an effective solution to the problem of poor efficacy of antibiotics against biofilms, which is particularly important considering the propensity of the major BRD bacteria to form biofilms.
- Assuming a suitable bank of phages for a given therapeutic target can be identified and purified with in vitro efficacy verified, the survival of these phage suspensions and their efficacy in the field will then need to be determined under field conditions.
- Phages can be administered concurrently with antibiotics to enhance their effectiveness.

During the last decade, many companies throughout the world, including Australia, have been investigating the potential therapeutic applications of phages (Housby, 2009). A more comprehensive understanding of the biology of phages has redefined research criteria appropriate to potential commercial application of phages in feedlot health. Phages can potentially play a very important role in the health management of feedlot cattle and further research is recommended.

Non-Specific Immunostimulants

Immunostimulants are compounds that stimulate the immune system by the activation and increased activity of any of its components. Specific immunostimulants, such as vaccines, stimulate antibody mediated immunity to specific antigens. Non-specific immunostimulants do not have antigenic specificity and they exert their effect by stimulating the innate or cell mediated immune system. Thus, non-specific immunostimulants increase the blood concentrations and activity of the phagocytic leukocytes - neutrophils and macrophages in the first instance, and later, T-lymphocytes. In addition, cell mediated immunity provides direction to the subsequent antibody dependent humoral immune response by the presentation of antigen, and is later involved with the removal of pathogens targeted by the humoral response. As shown by the period between initial vaccination and the development of protective blood concentrations of antibody, the humoral immune response has a lag of four to seven days. Therefore, enhancement of cell mediated immunity through the use of non-specific immunostimulants has the potential to increase the resistance of feedlot cattle to commonly encountered potential pathogens, and, to enhance the effectiveness of vaccines against specific pathogens. Non-specific immunostimulants will be referred to simply as immunostimulants hereafter.

There is some confusion between immunostimulants and immunoenhancers. Immunoenhancers are agents that contribute to the maintenance of a competent immune system through their roles in barriers to pathogen movement (eg. vitamins A and C), the identification and killing of pathogens (eg.

protein balance, trace element and vitamin status), and management of free radicals and toxic agents that arise as a consequence of inflammation aimed at combatting infections (eg. vitamins E and C, and the trace elements Se, Cu, Co, Mn, and Zn via their roles in various enzymes). Base tissue concentrations of immunoenhancers are essential for normal function, and in situations where pathogen challenge is increased, tissue concentrations of these greater than the requirement for the prevention of the clinical signs of deficiency can be beneficial. Immunoenhancers are covered elsewhere in this review.

A large number of compounds can act as immunostimulants and the effects of many have been studied in cattle, including bacterial fractions (Rogers et al., 2016; Kondracki, 1997; CzernomysyFurowicz and Furowicz, 1996; Dinsmore et al., 1995; Archambault et al., 1989), β-glucans from the cell walls of bacteria and fungi (Pedroso et al., 1996; Rubio et al., 1996; Paulik et al., 1993) viral fractions (Behrmann, 1995; Torrubia Diaz, 1994), the anthelmintics levamisole (Krasnikov et al., 1989; Chukwu, 1987; Ivanov et al., 1987; Krasnikov, 1986; Katrinka, 1985; Confer et al, 1985) and thiabendazole (Roth et al., 1984), immune complexes and various foreign proteins (Aizenshtein et al., 2013; Jung et al., 2009), and inorganic compounds such as the synthetic polyprenol, dihydroheptaprenol (Roth et al., 2002) and the xanthine derivative, pentoxil (Konopel'ko and Klimenkov, 1986).

An *in vitro* response in the activity of the polymorphonuclear cells (neutrophils and macrophages) that act as the first line of defence in cell mediated immunity to administration of any of the antigenic compounds listed above is inevitable in healthy animal tissues dependent on dose and is therefore largely meaningless to field applications. In addition, to prevent negative effects on animal health, an overt, systemic, clinical inflammatory response must not occur. Therefore, animal studies measuring gross animal health and production outcomes are the only meaningful measure of the effectiveness of immunostimulants and these will be discussed below for feedlot cattle or intensively reared calves. Further, only compounds non-toxic in the human food chain, and with zero or negligible residue risk, are worth investigating with commercial animal studies. Of the immunostimulants listed above, those with existing data justifying further investigation include bacterial fractions, β-glucans, and viral fractions. Levamisole is the potential immunostimulant that has been studied for the longest period and it will be discussed separately.
Registration studies to demonstrate efficacy with the bacterial DNA immunostimulant, Zelnate®, were presented at the 49th Annual Conference of the American Association of Bovine Practitioners (Nickell, et al., 2016). Using an experimental *Mannheimia haemolytica* challenge model these studies showed a significant reduction in lung lesions and mortality ($P < 0.05$). A subsequent large commercial study (n = 2589) evaluated the effects of Zelnate® administered concurrently with a respiratory viral vaccine (Pyramid 5®) either at feedlot arrival or with a 30 day delay to vaccination. At close out, treatment with the immunostimulant was associated with lower BRD mortality ($P = 0.06$) and lower total mortality ($P = 0.04$). Commercial field studies to evaluate the effectiveness of Zelnate® in the prevention of BRD in Australian feedlot cattle are warranted.

Pedroso et al. (1996) administered the β-1,3-glucan product, Evimunk®, both subcutaneously and intraperitoneally to 45 day old Holstein calves and found an enhanced cell mediated immune response at 14 days after treatment with both administration routes. This was followed by a commercial study with intensively reared calves on two farms with a history of BRD, where the treated group received 5 mg/kg Evimunk® subcutaneously at 30 days of age (Farm 1; 84 treated calves and 86 untreated controls: Farm 2; 210 treated calves and 219 untreated controls). Treatment with the

immunostimulant was associated with a reduction in the incidence of BRD. Further field studies are warranted with β-1,3-glucan products to verify this initial finding.

There are several immunostimulants that use fractions of different pox virus species, including Baypamun®, Duphapind®, Conpind® and Duphamun®). Torrubia Diaz (1994) found Duphamun® administered concurrently with a viral respiratory vaccine (Duphavac®, against infectious bovine rhinotracheitis, bovine viral diarrhoea and parainfluenza-3) enhanced cellular immunity and increased weight gain. On the other hand, Behrmann (1995) found Baypamun® did not reduce the incidence of BRD when administered as a blanket preventative and did not significantly improve BRD treatment outcomes. POLI-IF® is a combined immunostimulant which includes inactivated Newcastle virus and *Escherichia coli* endotoxin. A large commercial field trial (Galassi et al., 1996; 2782 treated calves and 2909 untreated controls) with the immunostimulant given twice at a seven to ten day interval in intensively reared calves subjected to the stresses of weaning, transport and crowding, found a reduction in morbidity, mortality and culls from all causes. This study (Galassi et al., 1996) notwithstanding, there is a lack of large commercial studies on the efficacy of pox virus based immunostimulants, and those products with some supporting evidence warrant further field studies.

Studies with calves have shown a positive response from the subcutaneous administration of levamisole simultanteously with vaccination in terms of increased antibody titres using vaccines against *Brucella* strain 19 (Chukwu, 1987), clostridial organisms (Katrinka, 1985), neonatal diarrhoea (Krasnikov et al., 1986), and parainfluenza-3 virus (Ivanov et al., 1987). Conversely, Confer et al. (1985) found simultaneous administration of strain 19 and levamisole did not alter antibody responses to *B. abortus*. Levamisole treatment enhanced cell mediated responses and decreased neonatal diarrhoea in calves (Krasnikov et al., 1986) but did not affect cell mediated responses in cattle treated with strain 19 (Chukwu, 1987). In summary, effects of levamisole on the stimulation of cell mediated immunity and antibody titre increase with concurrent vaccination are equivocal, and, large scale, commercial studies measuring the outcomes of animal health and production are lacking. The existing published studies do not support further investment in research into levamisole as an immunostimulant.

Recommendations for Future Research Into Alternatives to Antibiotics

It is recommended that the following issues be researched as part of a programme to reduce antibiotic use in feedlot cattle:

- ✓ Nitic oxide with concomitant measurement or manipulation of tissue concentrations of the antioxidants, vitamin E, glutathione peroxidase and vitamin C.

- ✓ Supplemental yeast or yeast products in cattle on high fibre backgrounding or starter diets.

- ✓ Direct fed bacteria with the establishment of lactate producing species in the intestine early in the feeding period and the inclusion of lactate utilising species throughout the feeding period.

- ✓ Evaluation of the availability and cost of malate in sufficient quantities to evaluate its effects in large, randomised commercial pen studies, possibly as an adjunct to the feeding of live cultures of lactate utilising bacteria.

- ✓ Phage identification and purification to produce commercial quantities of phage cocktails specific for the bacteria most commonly involved in bovine respiratory disease. Depending on initial results from this research, the next step would be the development of a kit and procedures for the rapid harvesting of the bacteria, most frequently involved in bovine respiratory disease and which live as commensals in the upper airways, for the rapid development of an appropriate phage cocktail.

- ✓ Large commercial field studies into the effectiveness of the immunostimulants, Zelnate®,
Duphamun®, POLY-IF®, and Evimunk®

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