REPRODUCTIVE DISEASE INVESTIGATION

GUIDELINES FOR VETERINARIANS

Northern Australia Biosecurity Surveillance Working Group
Version 1 April 2019

The Northern Australia Biosecurity Surveillance Project is part of the Australian Government's Agricultural Competitiveness White Paper, the government's plan for stronger farmers and a stronger economy.

Table of Acronyms

BCS	Body Condition Score
BEF	Bovine Ephemeral Fever
BHV	Bovine Herpes Virus
BSL	Biosecurity Sciences Laboratory Queensland
BTV	Bluetongue Virus
BVDV	Bovine Viral Diarrhoea Virus
BVL	Berrimah Veterinary Laboratories Northern Territory
CFT	Complement Fixation Test
DDLS	Diagnostics and Laboratory Services Western Australia
EAD	Emergency Animal Disease
ELISA	Enzyme Linked Immunosorbent Assay
EMAI	Elizabeth MacArthur Agricultural Institute New South Wales
IBR	Infectious Bovine Rhinotracheitis
Lepto	Leptospirosis
L1	First Lactation
MAT	Microscopic Agglutination Test
NABS	Northern Australia Biosecurity Surveillance
NABSnet	Northern Australian Biosecurity Surveillance Network
NATA	National Association of Testing Authorities
PCR	Polymerase Chain Reaction
PI	Persistently Infected animal with BVDV
PTIC	Pregnancy Tested In Calf
P4M	Pregnant within 4 Months of Calving
RBT	Rise Bengal Test
SAT	Slide Agglutination Test
Trich	Trichomoniasis
Vibrio	Vibriosis
VNT	Virus Neutralisation Test
VTM	Virus Transport Media

The purpose of this guide is to provide information for veterinarians which can assist with identifying whether a northern Australian beef herd has low reproductive performance and provide guidelines for how to investigate whether reproductive disease is a cause.

A key objective of the guide is to outline a simplified process for investigating whether reproductive disease is contributing to low reproductive performance, provide key differential diagnoses, outline investigation timeframes, laboratory tests available and costs and to develop resource information that veterinarians can use when recommending prevention and control measures to producers following a reproductive disease investigation.

Another focus of the Northern Australian Biosecurity Surveillance Network (NABSnet) of veterinarians is to increase supporting evidence for freedom of notifiable exotic diseases. This information is not only required to verify the property of origin disease status for live export but is used to support Australia's free status during negotiations with existing and new trading partners. Key notifiable exotic diseases to include as differentials in reproductive disease investigations include *Brucella abortus*, Bovine Herpes Virus-1 (abortion causing strains), Bovine Viral Diarrhoea Virus (BVDV) Type 2, exotic strains of Bluetongue (BTV) virus or circulating BTV strains causing clinical disease and any other diseases causing reproductive symptoms which are not known to occur in Australia.

Outline of Guide

Acknowledgements This guide is based on information drawn from research projects relating to reproductive wastage in northern Australia. These projects include Bull Power, Cash Cow, Beef Consultative Research Corporation and Calf Alive. Scientists, veterinarians and producers contributing to these projects and authors of scientific papers referenced include, but are not restricted to, Geoff Fordyce, Brian Burns, Peter O'Rourke, Richard Holroyd, Michael McGowan, Kieren McCosker, Nigel Perkins, Alistair Henderson, Andrew Brown, Lee Taylor, Ian Braithwaite, David Smith, Kevin deWitte and Sandra Jephcott.

Northern Australia

For the purposes of this guide and NABSnet, the northern Australian beef herd is located north of the Tropic of Capricorn and includes all of the Northern Territory.

It is divided into the following regions based on beef breeding production potential, climate and geography:

- **Tropical north Qld** including Cairns and Normanton
- **Top End/Kimberley** area around Darwin, Katherine and Victoria River District of western Northern Territory and eastern Western Australia
- **North-west Qld** areas from Townsville to Cloncurry
- **Channel Country** of Qld which is the extreme west of Qld from the Qld and South Australian border to Mt Isa
- **Central-west Qld** is north of a line from Rockhampton to Longreach including prominent beef areas around Emerald and Clermont. The Mackay area usually falls into this region.
- **Barkly Tablelands**
- **The arid zone of the Southern Northern Territory**
- **Pilbara** of Western Australia.

The most recent large scale herd reproductive research project 'Cash Cow' utilised country types to categorise properties for analysis of herd reproductive performance and generate benchmarks. Properties with forested land types and fertile soils in the central and south-east regions of Queensland were differentiated into those outside (Southern Forest) and within the Brigalow belt (Central Forest). In northern areas of Queensland, Northern Territory and Western Australia, land types predominated by tree-less black soil downs (Northern Downs) which included parts of central west Queensland and Barkly Tableland were separated from forested land types with low-fertility soils (Northern Forest) which included Northern Queensland and Top End/Kimberley. Figure 1. shows the location of properties participating in the Cash Cow project by country type. The northern Australian regions have been allocated to the most relevant country type representing the region to enable properties to benchmark herd performance.

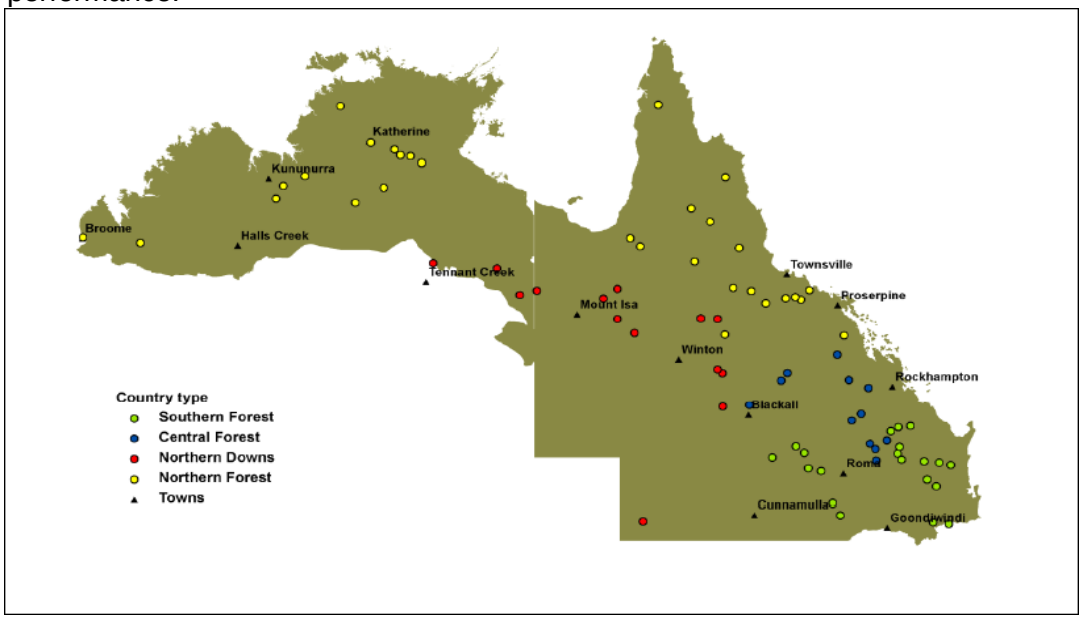

Figure 1. Map showing the approximate locations of country types for the northern Australian beef breeding production regions

Part 1. Reproductive Performance

1.1 Definition of low reproductive performance

Low reproductive performance in commercial beef breeding herds can be defined as:

- Delayed conception after females of an appropriate age and weight are exposed to bulls that have passed a veterinary fertility test, under adequate nutritional conditions
- Excess foetal/calf losses between pregnancy and weaning
- Mortality of pregnant or lactating cows

Low reproductive performance may be suspected when:

- Pregnancy rates fall below 80% for Cows or 85% for Heifers
- Branding rates fall below 70%
- Weaning rates fall below 65%
- The number of foetuses found exceeds 1%
- The number of dead calves found exceeds 2%

1.2 Assessment of reproductive performance

When assessing a property for reproductive performance, the performance and production parameters need to be benchmarked against other properties with **similar country types** or **historical performance** of the property being investigated under similar seasonal and management conditions.

Reproductive performance (median achievable level) for specific country types across northern Australia are outlined in Table 1. The range displayed in brackets is the 25th and 75th percentile figures respectively. The 75th percentile figure indicates the achievable level of performance that producers should be working towards for pregnancy testing rates and the 25th percentile figure for foetal/calf loss. The figures are based on properties participating in the Cash Cow project. Cash Cow was initiated because the causes of poor reproductive performance in northern Australian beef herds are multi-factorial and quantification of the impact of individual factors on performance of breeding mobs was lacking. Percentage of lactating cows pregnant within four months of calving (P4M), annual pregnancy rate, percentage foetal/calf loss between pregnancy diagnosis and weaning, and annual percentage of pregnant cows missing (mortality) were used to define performance. Four country types were used in the study which have been approximated to correspond with the northern Australian regions. Representative figures are currently limited for Channel country, Arid Zone and Pilbra regions.

These figures can be used for comparison with an individual properties annual pregnancy rate, foetal/calf loss and breeder mortality to determine whether there is low reproductive performance in the herd which may require further investigation. Pregnancy rate is the annual percentage of pregnant cows in a management group that are pregnancy tested in calf (PTIC) in a one year period. Foetal/calf loss is identified when a heifer or cow is diagnosed as pregnant in one year and dry (non-lactating) at least one month after the expected calving month the following year. Mortality is estimated as annual percentage of pregnant cows missing without being culled.

Pregnancy rates should to be divided into pregnancy rates for:

- Heifers
- First lactation (L1) cows and
- Mature cows (preferably being not older than 10-12 years depending on breeding history).

Foetal/calf loss between pregnancy and weaning should be divided into loss from:

- Heifers
- L1 cows and
- Mature cows

Heifers and L1 cows can have higher, more variable losses due to dystocia, no previous exposure to reproductive disease, maternal inexperience leading to higher rates of mis-mothering and failure of transfer of passive immunity.

A good indicator of what is a commercially achievable level of performance is the number of lactating cows that became pregnant within 4 months of calving (P4M). This should be **>80%** in more fertile areas and **>50%** in the less fertile areas.

- The median achievable herd pregnancy rates are **85%** on commercial properties in fertile areas, **80%** in black soil areas and approximately **65%** in the less fertile, harder country.
- The median achievable herd foetal/calf losses are **7%** on commercial properties in fertile areas, **10%** in black soil areas and approximately **13%** in the less fertile, harder country.
- The median breeder mortality rates are **6%** on commercial properties in fertile areas, **7%** in black soil areas and approximately **12%** in the less fertile, harder country. A tool for calculating breeder mortality is available from Meat and Livestock Australia https://www.mla.com.au/extension-training-and-tools/tools-calculators/Breeder-Mortality-Calculator/

A realistic target weaning rate for tropically adapted cattle in areas of northern Australia with good beef breeding production potential, in average or better rainfall years, is 70-80%. Industry surveys suggest that the majority of beef breeding herds in northern Australia would fall below this mark, as the reported overall annual branding percentage is 63%.

Table 1. Median achievable level of performance for breeders in different country types

Measure	(Southern Forest)	Central Qld (Central Forest)	Barkly Tablelands/ West Qld and Kimberley (Northern Downs)	North Qld/Top End/Kimberley (Northern Forest)
Annual Pregnancy rate herd (%)	85 (76-92)	85 (79-92)	80 (75-90)	66 (55-73)
Heifers Annual Pregnancy rate (%)	89 (75-93)	80 (75-87)	87 (77-94)	67 (40-81)
First lactation cows (L1) Annual Pregnancy rate (%)	84 (68-91)	78 (67-85)	75 (47-86)	43 (21-72)
Mature and aged cows > 4 years old Annual Pregnancy rate (%)	87 (77-93)	88 (79-92)	82 (75-91)	66 (56-74)
P4M (%)* re-breed rate	78 (65-89)	81 (69-88)	76 (69-81)	26 (14-47)
Foetal/calf loss herd (%)	6 (2-10)	7 (5-10)	10 (5-15)	13 (10-19)
Heifers Foetal/calf loss herd (%)	9 (4-14)	10 (4-18)	15 (7-20)	16 (11-19)
First lactation cows (L1) Foetal/calf loss herd (%)	5 (1-7)	7 (4-11)	5 (4-9)	10 (5-14)
Mature and aged cows > 4 years old Foetal/calf loss herd (%)	5 (2-9)	6 (4-9)	7 (3-15)	14 (9-19)
Breeder mortality (%)				
First lactation cow (L1) mortality (%)	7 (3-10)	12 (3-17)	7 (4-9)	8 (6-9)
Mature and aged cow > 4 years old mortality (%)	8 (3-13)	6 (1-11)	7 (4-13)	12 (6-18)

**Definition of P4M is lactating cows that become pregnant within 4 months of calving*

1.3 Major risk factors contributing to low reproductive performance in northern Australian beef herds

In northern Australia, reproductive wastage is caused by multiple factors and is primarily associated with risk factors other than infectious disease. Producer understanding of what these risk factors are and what impact that each risk factor may have on reproductive wastage in their herd is critical prior to initiating a reproductive disease investigation.

Major property, management, nutritional, environmental and infectious disease risk factors affecting performance are outlined in Table 2. Strategies to address the non-infectious disease risk factors to maximise reproductive performance are outlined in Appendix 1 of the Producer Guide

Three diseases (Bovine Viral Diarrhoea Virus (BVDV), Vibriosis and Botulism) are considered to be a moderate risk leading to greater than 5% reproductive wastage. Three diseases (Neosporosis, Akabane and Leptospirosis) are considered to be a low risk contributing to less than 5% reproductive wastage. Five other diseases (Trichomoniasis, Tick fever, Tetanus, Bovine Herpes Virus (BHV) and Bovine Ephemeral Fever (BEF)) are also considered to be low risk. Although the diseases are not thought to significantly contribute to reproductive wastage in a herd, they have been identified as priority endemic diseases (Lane, 2015) with some having a medium to high economic impact (Sackett *etal*., 2006).

Table 2. Major risk factors ranked by level of reproductive wastage

Highest risk factors (>20% increase in reproductive wastage)	**Moderate risk factors** (>5% increase in reproductive wastage)	**Lowest risk factors** (<5% increase in reproductive wastage)
INFECTIOUS DISEASE RISK FACTORS		
	• BVDV (incl exotic) • Vibriosis • Botulism	• Neosporosis • Akabane • Leptospirosis • Trichomoniasis • BHV (incl exotic) • BEF • Tick fever • Clostridial diseases
PROPERTY, MANAGEMENT, NUTRITIONAL AND ENVIRONMENTAL RISK FACTORS		
• Teat/udder conformation • Vit A deficiency	• Body Condition Score < 3 mid pregnancy • Mustering efficiency <90% • Mustering first lactation cows within 2 months of calving month • No dry season segregation based on foetal age • Breeder mob size > 800 head • Cows age >10 years of age • Dystocia • Cows calving between June and September • Low birth weight and low calf vigour • Country type • Low herd phosphorus	• Cows with large frame score (hip height >140 cm) • Mustering mature cows within 2 months of calving month • Failed to lactate in year after diagnosed pregnant • No follow up rain within a month after first storms at end of dry season • Pasture available <2 tonnes/ha in early dry season • Low protein dry season feed, ie crude protein CP:DMD dry matter digestibility ratio < 0.125 • Temperature humidity index (THI) >79 for >2 weeks in month of calving • Wild dog predation • Dehorning

1.4 Causes of reproductive wastage

Reproductive wastage can occur at any time of the reproductive process. It is defined as the proportion of animals within a stage of the reproductive cycle that do not advance to a nominated subsequent stage. Figure 2 outlines a flowchart to examine the potential infectious diseases that may be contributing to reduced reproductive performance. It is important to note that reproductive wastage is caused by multiple factors and is primarily associated with risk factors other than infectious disease. These risk factors are discussed in more detail in the Producer Guide.

The key events in the reproductive process when reproductive wastage occurs include:

- **Infertility/embryonic mortality**
- **Abortion**
- **Neonatal mortality**
- Pre-branding loss
- Post-branding loss
- Cow mortality

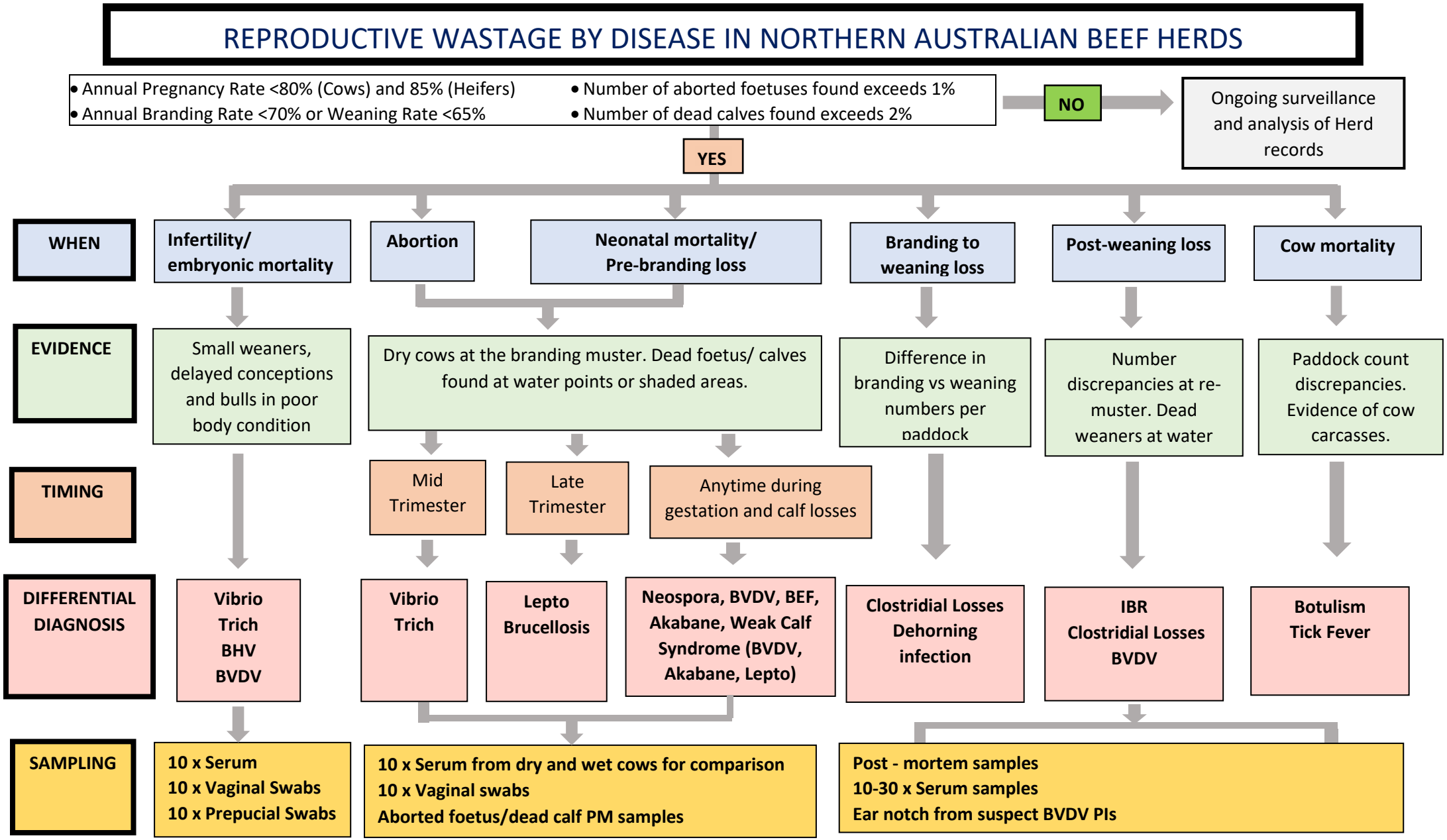

Figure 2. Flowchart to examine the potential infectious diseases that may be contributing to reduced reproductive performance

Table 3. Key events in reproductive process and the measure and potential cause of reproductive wastage

Event in Reproductive Process	Reproductive wastage	Cause
Infertility/Embryonic mortality		
Embryonic loss (Day 0–45)	• 10% but could be higher depending on number of bulls infected and unvaccinated /naïve females. • Delayed conception patterns leading to smaller weaners	• Vibrio and less commonly Trich
Delayed conception and low annual pregnancy rates and re-breed rates. This is particularly seen in heifers and lactating cows. In lactating cows, the age group most commonly seen with low re-breed rates are first lactation (L1) cows.	• 20 – 30% reduction in conception and or delayed conception so heifers are calving at wrong time of year nutritionally • Up to 40% reduced conception. Out-of-season (July – September) calving can have 50% lower re-breed rates than cows calving in October – December. • 10% but could be higher depending on number of bulls with low fertility and level of disease. • High grade Brahmans have 10% lower re-breed rates than cows with <50% Brahman • First lactation cows can have up to 30% lower re-breed rates than mature breeders	• Vibrio, Trich, BEF, BVDV, BHV • Age at puberty and joining weight • Low bodyweight and body condition score (BCS) cows • Grazing management, P status, month of calving
Abortion		
Foetal abortion (Day 45 – Birth)	• 10% but could be higher in epidemics	• Vibrio, Trich, BEF, BVDV, Lepto, Neospora, Akabane, Brucellosis
Calf born dead	• 10% but could be higher in epidemics	• BVDV, Lepto, Neospora, Akabane
Post-natal Calf Death		
Calf loss (Day 0-30)	• Up to 10%	• BVDV, Lepto, Neospora, Akabane • heat stress, cow BCS and predation (wild dogs)
	• Up to 10%	• Mustering time, method, handling
	• Up to 20%	• Cow culling policy e.g. calf rearing ability including low udder structure and bottle teats

Event in Reproductive Process	Reproductive wastage	Cause
Pre-branding Loss		
Loss between –Calving - Branding	• Up to 10%	• Dystocia
	• Up to 10%	Mis-mothering/low mothering ability /calf vigour
From Day 0-30, calves are highly susceptible to infection, dehydration and death.	• 10% but could be higher in epidemics	• Infectious diseases – outlined above
	• Up to 5%	• Predation (wild dogs)
	• Up to 10%	• Mustering time, method, handling
	• Up to 7%	• High heat stress during time of calving
	• Up to 20%	• Cow culling policy, ie calf rearing ability including low udder structure and bottle teats
	• Up to 10%	• Grass availability (<2 tonnes/ha at beginning of dry season), P deficiency, grass quality. This all can result in low BCS
	• 4% higher foetal / calf loss in subsequent years.	• Cow lactation status in the previous year e.g. lost a calf or foetus in the previous year
		• Inherited defects, stress, toxins
Post-branding Loss		
Calf loss (Branding-Weaning)	• 2-5%	• Clostridial diseases and dehorning/castration infection
	• Up to 10%	• Mustering time, method, handling
	• Up to 10%	• BVDV, mis-mothering, heat stress
Cow Mortality		
Cow / heifer mortality while pregnant or lactating	• Up to 25% unvaccinated/non-immune	• Botulism and tick fever
	• Up to 10%	• Low BCS, particularly pregnant cows
	• Up to 10%	• Dystocia
	• 6%	• Cow age
	• Up to 100%	• Toxins such as urea poisoning or poisonous plants

1.5 Information for Reproductive Disease Investigation

The veterinarian must source accurate and detailed information from the producer in the initial stages of an investigation for reproductive disease. The information may indicate that management changes for non-disease factors should be the implemented and the effect on reproductive performance measured before the collection of samples for reproductive disease testing is initiated. The Producer Guide outlines the necessary information required by the veterinarian for the investigation which includes:

- Producer Questionnaire which identifies epidemiological herd information
- Herd Records - different levels of herd records can influence the diagnostic outcome

The basic records needed to determine the cause of reproductive wastage include:

- pregnancy testing - foetal ageing, lactation status (wet/dry) to calculate calf loss and body condition score (BCS)
- cattle numbers by class (branders, weaners, heifers, first lactation cows, older cows) and average weight annually
- number of stock sold and purchased by class and average weight

Producer Questionnaire

A questionnaire completed by the owner/manager provides detailed herd information which may not be readily available in herd data records and is directly relevant to the current situation being investigated.

A template is available in the Producer Guide - Producer Questionnaire to collect relevant information for a reproductive disease investigation.

Ask the producer to complete this prior to the property visit to collect samples if possible, particularly for pastoral properties which you do not routinely visit for pregnancy testing and herd health.

Herd data records

Herd records provide accurate data to assist producers and other advisors in making herd management decisions. Some pastoral properties maintain electronic records and collect individual cow data crush side. Others record hard copy data crush side and transfer data to electronic form in the office. Some properties may not have detailed individual animal records but maintain a management diary.

Insufficient records can directly impact the outcome of a reproductive disease investigation. Major challenges to assessing the reproductive performance of extensively managed breeding herds include the reality that breeding females are often only mustered once or twice a year and herds are either continuously mated or employ long joining periods. Determining whether a pregnant female has reared a calf is an assessment of her lactation status at the time of each round of weaning but this also depends on individual identification and recording the information either electronically via NLIS tags or manually.

Herd records are essential to enable the assessment of the impacts of management, nutritional, environmental and infectious disease factors on:

- how efficiently cows become pregnant,
- the likelihood of pregnant cows rearing a calf and
- the likelihood of cow mortality

Part 2. Reproductive Disease investigation

Foetal and calf loss between pregnancy testing and weaning is a major problem in northern Australia. A high proportion of loss occurs within a week of birth (neonatal mortality). Reasons for loss are numerous. Although the specific causes of the majority of loss remain unconfirmed, recent research indicates that the primary causes for loss are not infectious diseases, but are nutritionally related *(Letchford, P. pers comms 2018).* Although under-nutrition is not a specific disease, it is an important and widespread predisposing cause of many of the more commonly seen diseases in beef cattle in northern Australia, sup-optimal reproductive performance and predisposing factor for dystocia. Incorporating a complete reproductive wastage differential diagnosis including non-infectious causes is now a recommended practice for veterinarians investigating low reproductive performance which may be associated with reproductive diseases.

2.1 Steps involved in Reproductive Disease Investigation - Vet

When a reproductive disease problem in a herd is suspected by a producer, there are benefits to having an established client relationship with the producer and knowledge of the current and previous herd management. However, the following steps outlined as a Standard Operating Procedure (SOP) will assist with new clients and where there is a lack of historical knowledge of the reproductive performance of the herd.

Standard Operating Procedure (SOP)

1. Assess the current reproductive performance of the herd and confirm that the property's reproductive performance is **lower** than what can be expected for different classes of stock in similar country (Refer to Table 1).
2. Ask the producer to:
    - complete the Producer Questionnaire
    - source detailed Herd Records relating to the stock numbers and reproductive performance
3. Review the non-disease risk factors (**management, nutrition, environment** and **animal risk factors** of the herd. (Refer to Table 2).
4. If management, nutrition, environment or non-disease animal factors are contributing to the problem, discuss with the producer how this can be **resolved** (Refer to Producer Guide).
5. If disease is suspected to be the cause or contributing to the problem, identify the likely causal **diseases** in differential diagnosis (Refer Figure 2)
6. Discuss with producer which **classes of stock**, e.g. bulls, heifers, first lactation cows, mature cows, cows from a particular paddock or particular purchase group need to be tested, how many and estimated cost for investigation and set a **date** for sampling (Refer to Table 4)
7. **Contact the veterinary laboratory** to confirm specific disease testing requirements and costs and source sampling and packaging equipment.(Note that samples may need to be sent to different laboratories for specific testing) (Refer to Appendix 1)
8. **Collect samples** and dispatch them on the date arranged and advise producer of the estimated time frame for results.
9. Prepare **Disease Investigation Report** for producer after receiving results from laboratory outlining:
    - what the disease test results are and what they mean
    - what impact the disease/s may have on the herd over time
    - what actions need to be taken to reduce the impact of the disease or eliminate it if possible

- what preventative or control measures can be undertaken and the cost benefit of implementing these measures.

10. Producer decides whether to implement recommendations. Maintain contact with producer to determine the **outcomes** of any control measures introduced and reassess further actions.

2.2 Reproductive Disease Laboratory tests, sampling and transport

The Guide for Reproductive Disease Lab Tests and Costs (Appendix 1) outlines the reproductive disease testing available and costs for each of the State Veterinary Laboratories for northern Australia. It also provides information relating to ordering test kits and media and relevant forms for each laboratory. Test availability and prices are subject to change at any time, so laboratories should be contacted to confirm test availability and costs when initiating investigation or ordering testing kits and media.
Samples tested for **exotic notifiable diseases** (BVDV-2, BHV1.1 and 1.2a, Brucellosis (*Brucella abortus)* and exotic Bluetongue virus serotypes are free of charge at State Veterinary Laboratories.

Most laboratories charge for other reproductive disease tests including Vibriosis, Trichomoniasis, BVDV-1, Leptospirosis, Neosporosis, Infectious Bovine Rhinotracheitis (IBR), BHV1.2b, Akabane, BEF and other diseases. The pathologist may also determine further testing is warranted.

The NABSnet **Significant Disease Investigation (SDI) Guide** http://nabsnet.com.au/sdi-field-guide/ provides further details on sample collection and packaging, laboratory transport and biosecurity.

- **Sampling**

In northern Australia, the extensive pastoral system and limited or challenging access to cattle during infertility/abortion events means that veterinarians often do not collect samples at the critical time. Diagnosis is usually based on retrospective/convalescent serology taken from a representative sample of the herd several months later. This sampling technique is usually better at demonstrating that an agent is not the problem than verifying that infectious disease was the cause of the problem.

A lower than expected level of reproductive performance is usually not apparent until after pregnancy testing and branding/weaning when herd data records are reviewed. Therefore, some veterinarians routinely collect vaginal swabs and serum from breeders with symptoms consistent with infertility during pregnancy testing.

Samples for diagnostic testing should be collected when cattle are available. Table 4 shows the basic samples to collect during a reproductive disease investigation from different classes of stock. Figure 2 should be used to identify the list of differential diseases to include on the laboratory submission form. The pathologist will finalise the diagnostic testing.

- **Aborted foetus/calf loss**

Under normal extensive conditions it is very rare to find aborted foetuses in the paddock due to predation. Foetal remnants are most commonly seen in yards or on trucks following transport. It is critical that the aborted foetus or dead calf is collected for examination immediately and the associated heifer/cow is identified and held in the yard or following transport are also available for sampling. Appendix 2 provides further detail on investigating abortions and neonatal mortalities.

- **Samples from bulls**

Opportunity for sampling bulls usually occurs when bulls are being examined prior to joining or during mustering for other management purposes.

- **Samples from heifers and cows**

It may become apparent during pregnancy testing that there may be a problem e.g. lower than expected pregnancy rate, delayed pregnancy, evidence of infection or reabsorbing foetal remnants. If the pregnancy testing is being undertaken by a veterinarian, then samples for disease testing can be taken at the same time or cattle drafted off and held in yards until sampling equipment can be sourced.

If the pregnancy testing is being undertaken by a producer, then contact will need to be made with a veterinarian to discuss the investigation and potential sampling options. Most producers will not have access to the sampling equipment required for a reproductive disease investigation.

Where veterinarians routinely collect samples during pregnancy testing, the samples may be submitted to the laboratory following analysis of the pregnancy testing results showing low performance or they may be submitted the following year if the mob's pregnancy or calving results are significantly different. Alternatively, samples can be taken from the same mob in consecutive years to give comparative results.

Table 4. Reproductive Disease Investigation sample collection guide for different classes of stock

Class	Sample	Packaging & Transport	Reproductive Disease DDx
Foetus/Calf	• Foetus and whole placenta or carcass • Fresh and fixed tissue - liver kidney lung brain heart spleen, skeletal muscle • Pericardial, pleural or peritoneal fluid	• Chilled – refrigerated or esky and ice • Fixed tissue in 10% formalin	• Neospora • Akabane • Brucellosis • BVDV • Lepto
Bulls	• 10 x Tricamper preputial scrape/wash (use Campy culture kits and Trich PCR kits) • 10 x serum	• Trich PCR (keep chilled) • Vibrio culture (keep at room temperature) • If both, keep at room temperature • Chilled – refrigerated or esky and ice	• Trich • Vibrio
Heifers/L1 and Mature Cows	• 10 x Tricamper vaginal scrape/wash (use Trich PCR kit) • 10-20 x vaginal mucous swab (use Camp ELISA kit) • 10-20 x serum	• Trich PCR or culture (room temperature) • Vibrio ELISA (chilled) • Chilled – refrigerated or esky and ice	• Trich • Vibrio • Lepto • Neospora • BVDV • Akabane • BHV • Brucellosis
Weaners	• 10 x serum • Ear notch for suspect BVDV PIs • If there is respiratory disease, nasal swab in VTM for IBR	• Chilled – refrigerated or esky and ice	• BVDV • IBR

Sample each management group and each age group (heifers, L1 and mature cows) if mixed ages within the mob

2.3. Diagnosing Reproductive Disease

Accuracy of laboratory results is directly dependent on quality of samples submitted. A poor quality sample is more likely to result in false negative than false positive results. Serology, used for detecting antibodies, is more robust than culture or molecular tests used for detecting organisms.

At the laboratory, the pathologists will review the information provided on the laboratory submission form, the appropriateness of samples submitted and differential diagnoses listed and tests requested. A pathologist may recommend alternate testing and suggest alternative diseases to be further investigated.

It is essential that veterinarians have the ability to contact pathologists at State Veterinary Laboratories to provide advice on the most appropriate samples to collect, how to collect them, ideal time period to transport samples to the laboratory and to assist with interpretation of results. When in doubt, always contact the laboratory to confirm most appropriate methods which will increase the chance of getting a meaningful results.

Vibriosis and Trichomoniasis

In bulls *Campylobacter fetus subspecies venerealis* and *Tritrichomoniasis foetus* inhabit the mucosa of the glans penis, prepuce and the distal portion of the urethra. Preputial scrapings and preputial washings are suitable for culture. Bulls commonly become persistent carriers of these organisms and are the main reservoir of infection in the herd. A persistent carrier stage in cows is uncommon.

In cows*, T. foetus* may colonise the vagina for about a month after infection, and *C. fetus venerealis* may colonise the vagina for a few months after infection. Infertility is more common than abortion, but when abortion does occur, organisms may be cultured from uterine discharges and the abomasal contents of aborted foetuses.

The diagnosis rate of Vibriosis and Trichomoniasis infections is lower in cows than bulls, since vaginal infection is usually eliminated a few months post infection. The Campylobacter ELISA test, which detects antibodies in vaginal mucus, is usually a more reliable test to check for evidence of Campylobacter infection in cows.

The epidemiology of the disease is not fully understood as sensitive and specific tests for both the bacterium and evidence of infection are currently not available.

Vibriosis

- In an unvaccinated herd, sample suspect and normal cows using ELISA and test herd bulls using PCR or culture. Vibriosis screening of bulls using PCR prior to introduction to the herd may be considered. PCR testing is conducted at AgriBio Veterinary Diagnostic Services Laboratories Victoria.
- In at least 10 affected heifers/cows, sample vaginal mucus into specialised media for ELISA to detect agent specific IgA. Samples for **ELISA** should be stored and transported at 4^0C.
- In 10 bulls do a preputial scraping/wash into specialised culture transport media which must be obtained from the laboratory prior to testing. **Media** should be stored 4^0C at and used before expiry dates.
- For aborted foetuses and dead calves, collect the placenta or foetal abomasal contents for culture.
- It is recommend that the **Campylobacter culture** media is transported to the laboratory within 2 days and remains at room temperature (18 – 37^0C). As this

transport time is challenging for most northern Australian properties, culture may not be practical.

- **Campylobacter ELISA** vaginal swabs can be transported to the laboratory > 2 days. The ELISA can be used as an individual animal test for abortions within the previous 1-3 months and as a herd screening test for infertility. In herd tests, low numbers of test positives can be difficult to interpret but large numbers of test positives indicate infection was present in the herd. Immunity lasts 3 – 4 years, so positive herd results doesn't necessarily mean recent infection. Test specificity remains questionable.

Trichomoniasis

- Sample herd bulls for Trichomoniasis PCR or culture.
- In 10 bulls do a preputial scraping/wash into specialised culture transport media which must be obtained from the laboratory prior to testing. **Media** should be stored at 4^0C and used within 6 weeks.
- For aborted foetuses and dead calves, collect the placenta or foetal abomasal contents for culture.
- It is recommended that the **Trichomoniasis culture** media is transported to the laboratory within 2 days and remains at room temperature (18 – 37^0C). For this reason, culture may not be practical.

Leptospirosis

Cows may only stay seropositive for a few weeks after abortion. Depending on the serovar, test sensitivity maybe as low as 40% so veterinarians are required to test at least 10% of the herd to get a meaningful result. *L. hardjo* is not well documented as a cause of major reproductive problems in cattle in Australia. *L. pomona* may cause abortion storms. Pathologists recommend not to bother with convalescent serology unless the veterinarian is highly suspicious that leptospirosis caused the problem and abortion was within the last few weeks.

Many studies in northern Australia have shown that the prevalence of Leptospirosis infection in unvaccinated herds is surprisingly very low even when co-operating properties experienced higher than average rainfall during testing periods. Median mob seroprevalence for both *L. hardjo and L. pomona* is typically <20%, with fewer than 10% of mobs showing evidence of recent infection. This result could be because leptospirosis immunity is short-lived. There is a trend for higher foetal/calf loss in mobs that have evidence of widespread recent infection with the pig-adapted serovar, *L. pomona.*

There appears to be a higher prevalence in southern and central Queensland and some veterinarians recommend annual vaccination of heifers with 7-in-1 containing *L. hardjo and L. pomona*. In contrast, there appears to be a low prevalence in the Northern Territory and Kimberley/Pilbara regions. This difference may be due to the fact that testing on smaller commercial properties is undertaken closer to calf loss whereas testing is done on larger northern properties months after it has occurred.

Tests that detect the presence of bacteria (PCR and culture) will only yield positive results in the acute/bacteraemic phase. Tests that detect antibodies will produce positive results from 6-8 days from the onset of illness for immunoglobulin M (IgM) enzyme-linked immunosorbent assay (ELISA), and from 10-12 days from illness onset for microscopic agglutination test (MAT).

- In an unvaccinated herd, collect serum samples from 10 suspect and 10 normal heifers/cows for Leptosporosis MAT. The common serovars tested are *L. pomona*, *L. hardjo* and *L.tarasovvi*
- For aborted foetuses and dead calves, the whole foetus and placenta could be collectd and chilled for PCR/culture and direct examination of leptospires. For practicality and transport, foetal kidney and urine from the associated cow for PCR is sufficient.
- Serum and fresh samples should be stored and transported at 4^0C.

Neosporosis

Neospora.caninum infection is widespread in northern Australian beef herds. Wild dogs, the primary host are commonly infected with this parasite. It appears a majority of infections are vertical (cow to calf during pregnancy) although horizontal infection from a wild dog prior to first mating is also possible; both of these infection sources are related to low abortion rates. Antibodies in cows may decrease within weeks of an abortion or persist. Seropositivity in a cow does not confirm it aborted due to Neospora although a seropositive cow is more likely to have aborted/delivered a weak calf due to Neospora than a seronegative one. Sero-survey could be meaningful if sufficient number of cows with or without calves are compared. Mummification is common.

- Collect serum samples from 10 suspect and 10 normal heifers/cows for Neospora ELISA.
- For aborted foetuses and dead calves, collect the whole foetus and placenta chilled or fresh (brain, heart, liver) and fixed (brain, lung, heart, skeletal muscle, liver, lymphoid tissues and adrenal gland) tissues for Ag detection in brain
- Serum and fresh samples should be stored and transported at 4^0C.

Bovine Viral Diarrhoea Virus (Pestivirus) (BVDV-2 is exotic notifiable disease)

Guidelines for BVDV Testing for Veterinarians outlines information on BVDV sampling, diagnostic tests and management strategies.

- Collect serum samples from 10 suspect heifers/cows for BVDV AGID for Ab
- In aborted foetuses and dead calves, collect fresh (lung and spleen) for BVDV Ag using Ag-capture ELISA or BVDV virus using PCR or virus isolation and fixed tissues for histopathology
- Pericardial fluid to test IgG BVDV Ab
- Collect serum or ear notch from suspect persistently infected (PI) animal for BVDV Ag-capture ELISA
- Serum and fresh samples should be stored and transported at 4^0C.
- Interpreting serology and recommending a management plan can be difficult due to the complex nature of the disease. AGID may be misleading in its ratings as it only shows recent infection if blood is taken within 6 weeks of infection. If cows were infected at the time of joining in December-January and are not bled until first round, it will not be shown as recent infection.

Akabane

In a cow infected while pregnant the virus can affect the nervous system of the foetus, causing abortions, stillbirths and deformed calves. The signs seen in the calves will depend on the stage of pregnancy at which they were infected with hydranencephaly (3-4 months), arthrogryposis (4-6 months) and encephalopathy (8-9 months). These calves frequently cause dystocia. When born alive they are usually small, underweight, weak and unable to stand.

- Collect serum samples from 10 suspect heifers/cows for Akabane ELISA
- In aborted foetuses and dead calves, collect fresh (brain) and fixed (brain, spinal cord, muscle) for Akabane ELISA, VNT or PCR and fixed tissues for histopathology
- Serum and fresh samples should be stored and transported at 4^0C.

Bovine Herpes Virus (BHV1.1 and 1.2a are exotic notifiable diseases)

Abortion is usually associated with the respiratory form of the disease when a non-immune pregnant heifer or cow is infected with BHV-1. The virus crosses the placenta and causes organ necrosis in the fetus and subsequent abortion. Exposure of multiple susceptible animals in a herd can result in abortion storms, with as many as 25%-60% of cows in a herd aborting. BHV-1 abortions may occur at any gestational stage, but are most common between 4-8 months of gestation. Abortion is currently only associated with virulent subtypes of BHV-1 which are not known to be present in Australia. BHV-1 can also cause a generalized disease in newborn calves, characterized by enteritis and death. BHV-5 and BHV-6 have also recently been implicated as a cause of venereal disease in southern beef herds.

- Collect serum samples from 10 suspect heifers/cows or bulls for BHV VNT
- In aborted foetuses and dead calves, collect whole foetus or fresh (kidney and adrenal glands) for immunohistochemistry
- Serum and fresh samples should be stored and transported at 4^0C.

Brucellosis (*B. abortus* is exotic notifiable disease)

Bovine brucellosis causes mass abortion in cattle herds, with affected cows remaining carriers and suffering continuing reproductive problems. In a susceptible herd, an outbreak of bovine brucellosis would be expected to be seen as a storm of abortions after the fifth month of pregnancy. Infections may also cause stillborn or weak calves and retained placentas. In the bull, the disease may cause inflammation and swelling of one or both of the testicles. Bull fertility is severely reduced during the acute phase of the disease, but may regain fertility if one of the testicles is unaffected; these bulls can potentially spread the disease.

- Collect serum samples from 10 suspect heifers/cows for RBT, CFT, ELISA or SAT
- Collect vaginal mucus, uterine discharge or semen for culture (referred to Reference Laboratory)
- In aborted foetuses, collect the whole foetus and placenta chilled or fresh (liver, lung, spleen, heart blood, abomasal contents, pericardial, thoracic or peritoneal fluid)
- Serum and fresh samples should be stored and transported at 4^0C.

Part 3. Best Management Practice

3.1 Disease Investigation Report

A report which outlines the basic history, laboratory results, interpretation of results, diagnosis or probable cause and key recommendations for treatment or control of the current event and future prevention is an important document to conclude the investigation.

The benefits of a disease investigation report include:

- Simple overview of the investigation outcome
- Requirement by the owner to justify the expense of investigation and management strategies implemented
- Source of historical information for future investigations
- Professionalism

For NABSnet subsidised SDIs, a report is a requirement for payment.

3.2 General recommendations

The Northern Beef Program provides key recommendations to achieve optimum reproductive performance in northern Australian beef herds with a Heifer Program focused specifically on maximising heifer performance. These programs can be used in discussions with producers with selection of target actions for the producer to implement.

3.3 Reproductive Disease Recommendations

The strategy of disease control and prevention will depend on the producer's attitude to risk and the cost benefit of using the selected preventative measure. It is also vital to demonstrate the effect of the intervention, so measuring and comparing reproductive wastage before and after the application of the intervention is important to demonstrate the effect to the producer. The following recommendations are simple best practice measures for maintaining and improving reproductive performance.

- Monitor the reproductive performance of the herd against the median achievable level for properties in similar country (Table 1). A Reproductive Disease Investigation can be initiated where a herd has lower than expected performance, bulls, heifers or cows demonstrate symptoms consistent with reproductive infectious diseases during bull testing or pregnancy testing or aborted foetuses or deformed calves are found.
- Maintain sufficient herd records to enable the herd performance to be assessed annually
- Undertake bull testing and pregnancy testing in the herd
- Understand the reproductive disease risk factors and apply preventative measures where budget allows:
    - Vibriosis vaccination for bulls and heifers if vibriosis is known to be a problem
    - Pestivirus strategy Guidelines for investigation and control of BVDV
    - Annual 7-in-1 vaccination for breeders (Leptospirosis and Clostridial diseases) or 5-in1 if warranted
    - Botulism vaccination strategy to reduce breeder mortality

  - Tick fever vaccination in marginal cattle tick areas and for new introductions to cattle tick areas.
  - BEF vaccination for new introductions of bulls from BEF virus free regions
- Test for exotic notifiable diseases to support property disease free status when undertaking other reproductive disease testing.

3.4 Reproductive Disease Factsheets

A number of simple information sheets for key reproductive infectious diseases are available to assist producers with understanding the important diseases, the potential impact on reproductive performance in the herd and what can be done to prevent it entering the herd or when it is already present within the herd. A relevant factsheet should be provided to the producer with the Disease Investigation Report where positive laboratory test results are received. The report should provide an interpretation of what the results mean.

The **Factsheets** outline the:

- Cause
- Northern Australian distribution
- Symptoms
- Reproductive impact
- Sampling and diagnosis
- Prevention and control measures

Akabane Factsheet

BEF Factsheet

Brucellosis Factsheet

BVDV Factsheet

Bovine Herpes Virus Factsheet

Leptosporosis factsheet

Neosporosis Factsheet

Trichomoniasis Factsheet

Vibriosis Factsheet

References

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McGowan M, Fordyce G, O'Rourke P, Barnes T, Morton J, Menzies D, Jephcott S, McCosker K, Smith D, Perkins N, Marquart L, Newsome T, Burns B (2014). Northern Australian beef fertility project: CashCow. Report prepared for Meat & Livestock Australia, Sydney. Available at: https://www.mla.com.au/research-and-development/search-rd-reports/final-report-details/productivity-on-farm/northern-australian-beef-fertility-project-cashcow/370

Sackett D, Holmes P, Abbott K, Jephcott S, Barber M (2006) Assessing the economic cost of endemic disease on the profitability of Australian beef cattle and sheep. Report prepared for Meat & Livestock Australia, Sydney. Available at: http://www.mla.com.au/Research-and-development/Search-RD-reports/RD-report-details/Animal-Health-and-Biosecurity/Assessing-the-economic-cost-of-endemic-disease-on-the-profitability-of-Australian-beef-cattle-and-sheep-producers/120

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APPENDIX 1 - GUIDE TO REPRODUCTIVE DISEASE TESTS AND COSTS IN NORTHERN AUSTRALIA STATE LABORATORIES

PLEASE NOTE: Test availability and prices are subject to change. Please contact the relevant laboratory for current advice

STATE LAB	QUEENSLAND	WESTERN AUSTRALIA	TERRITORY	NEW SOUTH WALES
Contact details	**BSL Qld** **Phone:** 07 3708 8762 **Email:** bslclo@daf.qld.gov.au **Address:** Block 12, 39 Kessels Road Coopers Plains QLD 4108	**DDSL WA** **Phone:** 08 9368 3351 **Email:** DDLS@dpird.wa.gov.au **Address:** C Block, 3 Baron-Hay Court South Perth WA 6151	**BVL NT** **Phone:** 08 8999 2249 **Email:** bvl@nt.gov.au **Address:** Makagon Rd, Berrimah NT 0828	**EMAI NSW** **Phone:** 1800 675 623 **Email:** laboratory.services@dpi.nsw.gov.au **Address:** Woodbridge Road, Menangle NSW 2568
Tests available and fees	https://www.business.qld.gov.au/industries/farms-fishing-forestry/agriculture/land-management/health-pests-weeds-diseases/sample-testing/submitting	https://www.agric.wa.gov.au/sites/gateway/files/Department%20of%20Primary%20Industries%20and%20Regional%20Development%20services%20products%20and%20fees%202017-18.pdf	https://dpir.nt.gov.au/primary-industry/laboratory-services/berrimah-veterinary-laboratory Prices for specific tests are not on the website	https://www.dpi.nsw.gov.au/about-us/services/laboratory-services/veterinary
Campylobacter culture (PCR AgriBio Vic)	Test kits $8.00/animal $51.23/animal	$76.50	Referred to AgriBio Vic for PCR or DDSL WA for culture	Test kits Campylobacter Enrichment Transport Media (CETM) **$5.50** $50.50/animal (Uses older transport media)
Campylobacter ELISA	Test kit $6.00/15 animals $15.43/animal https://www.daf.qld.gov.au/__data/assets/pdf_file/0017/133730/BSL-GEN-010-Bovine-infertility-sample-collection-ELISA.pdf	$24.00	Referred to EMAI NSW	Test kits PBST and sterile swabs – Freight only $20.75/animal
Trich PCR (Not NATA accredited)	Test kits $6.00/animal $68.45/animal	$75.00	N/A	Tricamper Sampling tool (pack of 10) **$79.50** **PBS – Freight only** **Test $120.25**

STATE LAB	QUEENSLAND	WESTERN AUSTRALIA	TERRITORY	NEW SOUTH WALES
Trich culture	Test kits $8.00/animal $51.23/animal	$70.00	Referred to AgroBio Vic	Trichomonas Foetus Enrichment Media (TFEM) **$10.75** **$41.00/animal**
BVDV AGID (Can transport for up to week)	$17.62	N/A	$11.55	$19.95
BVDV ELISA		$17.50	N/A	$15.25 $11.20 (> 10 samples)
BVDV Antigen Capture ELISA	$17.49	$17.50	35.30	
BVDV PCR	$68.45	$85.00	Referred to EMAI NSW	$84.55
BVDV VNT	$35.70	$34.25	N/A	$43.45
Lepto MAT *L.hardjo*, *L.pomona*, *L.tarassovi*	$14.02/animal per serovar	Referred to Forensic and Scientific Services Leptospirosis Reference Laboratory C/- Public Health Property Point, Loading Dock 1 39 Kessels Road Coopers Plains, Qld 4108	Referred to Forensic and Scientific Services Leptospirosis Reference Laboratory C/- Public Health Property Point, Loading Dock 1 39 Kessels Road Coopers Plains, Qld 4108	$13.00/animal per serovar
Leptospira sp. PCR	$68.45	$85.00	N/A	$87.50
Neospora ELISA	$14.02	$17.50	Referred to DDSL WA	$12.25
Akabane VNT	$35.70	$34.25	$28.00	$43.45
Akabane RT-PCR		N/A	N/A	$84.55
Akabane ELISA		$17.50	N/A	$12.25
Aino VNT	$35.70	$34.25	$28.00	$43.45
Aino RT- PCR		N/A	N/A	**$84.55**
BEF PCR	$68.45	N/A	$77.00	$84.55
BEF VNT	$35.70	$34.25	$28.00	$43.45
BEF ELISA		N/A	N/A	$12.25
BHV-1 VNT	$35.70	$34.25	$28.00	

STATE LAB	QUEENSLAND	WESTERN AUSTRALIA	TERRITORY	NEW SOUTH WALES
BHV 1.1 & BHV-1.2a	Referral	Referral	Referral	
BTV PCR	$68.45	$85.00	$77.00	$84.55
BTV VNT	Serotype 1 or 21 $35.70	N/A	$28.00 per serotype 1,2,3,5,7,9,12,15,16,20, 21,23	
BTV AGID	$17.62	N/A	$11.55	$19.95
BTV ELISA	$15.42	$17.50	$13.40	$12.25
Brucella sp. culture *B.abortus, B.suis*	N/A	Not routine	N/A	$64.75
B.abortus ELISA	N/A	N/A	N/A	$17.00
B.abortus CFT *B.suis* CFT	$13.22	$8.85	N/A	$13.00
Rose Bengal *B.abortus, B.suis*	$5.45	$8.85	$5.50	$6.25
B.abortus SAT	N/A	Not routine	N/A	$12.00
Fungal culture General	$52.54	$70.00	$44.30 Free for disease investigation	$50.50
Fungal culture Selective		$70.00	Free for disease investigation	$64.75
Chlamydia psittaci FAT	*Chlamydophila* spp. detection FAT $48.12	N/A	N/A	$67.71
Chlamydiaceae PCR	$68.45	$85.00	N/A	$87.50

Tests highlighted green are for national notifiable diseases and should be free of charge for suspect cases of notifiable disease reports

Request form for Repro Test Kits and Media

BSL Qld	https://www.daf.qld.gov.au/__data/assets/pdf_file/0006/313278/Equipment-Request-Form-GEN001.pdf
EMAI NSW	Test Kits https://www.dpi.nsw.gov.au/about-us/services/laboratory-services/kits-and-media
	Media https://www.dpi.nsw.gov.au/__data/assets/pdf_file/0006/680586/Media-request-form.pdf

Laboratory Submission Forms

Queensland BSL	https://www.daf.qld.gov.au/__data/assets/pdf_file/0006/65733/GEN008SpecimenAdviceSheet.pdf
WA DDSL	https://www.agric.wa.gov.au/sites/gateway/files/DDLS%20animal%20pathology%20submission%20form_0.pdf
NT BVL	https://dpir.nt.gov.au/__data/assets/pdf_file/0011/597431/BVL-san.pdf
NSW EMAI	https://www.dpi.nsw.gov.au/__data/assets/pdf_file/0007/680425/Vet-specimen-advice-form-Feb2018.pdf

Other Forms

Repro History Form	https://www.daf.qld.gov.au/__data/assets/pdf_file/0007/1258945/BSL-GEN-006-Cattle-Herd-Reproductive-History-form-1.pdf
Sample Numbering Sheet	https://www.daf.qld.gov.au/__data/assets/pdf_file/0003/53742/Laboratory-SampleNumberingInformation-GEN113.pdf
Necropsy Form	https://www.daf.qld.gov.au/__data/assets/pdf_file/0010/98884/interactive-necropsy-form.pdf

Instructions for Reproductive diseases sampling

Serological sampling (BSL Qld) https://www.daf.qld.gov.au/__data/assets/pdf_file/0009/1258947/BSL-GEN-005-Submitting-samples-to-the-Biosecurity-Sciences-Laboratory-for-investigation-of-infertility-and-abortion-in-cattle.pdf

Vibrio ELISA (EMAI NSW) https://www.dpi.nsw.gov.au/__data/assets/pdf_file/0004/800689/SCG-001-Diagnosis-of-Bovine-Venereal-Campylobacteriosis-Vibriosis-by-ELISA.pdf

Tricamper sampling (BSL Qld) https://www.daf.qld.gov.au/__data/assets/pdf_file/0004/277753/Tricamper-sampling-handout.pdf

Vibrio & Trich culture (EMAI NSW) https://www.dpi.nsw.gov.au/__data/assets/pdf_file/0010/800695/SCG-003-Campylobacter-and-Tritrichomonas.pdf

Handling Media for Vibrio & Trich culture (EMAI NSW) https://www.dpi.nsw.gov.au/__data/assets/pdf_file/0007/800692/SCG-002-MI-Camp-and-Trich-Culture.pdf

Video Vibrio & Trich sampling (CSU) https://www.youtube.com/watch?v=LUhUkg4jB-8

BVL Submitters Handbook (NT) https://dpir.nt.gov.au/__data/assets/pdf_file/0008/256769/submitters-handbook.pdf

APPENDIX 2 - INVESTIGATION OF ABORTION AND NEONATAL CALF DEATHS

The following is an indication of information that could be collected and would assist you and the pathologist in the diagnosis of what caused the abortion, foetal death or neonatal death:

1. **Duration of pregnancy**
2. **Epidemiological information** (Where possible, use objective measurements):
    - What is the abnormality
    - What is the apparent age at onset and age at death
    - What clinical signs are consistently associated with the problem
    - What is the prevalence and proportional risk in particular groups (maternal, paternal, nutritional, vaccinated, etc)
    - What is the parity of the dam(s) that gave birth to the animal(s) and what proportional risk does this reflect within the group
    - What is the birth history of affected animals (prolonged?)
    - Has there been any difference in management of the dams of the affected animals to the group as a whole
    - Environmental conditions at time of calving
    - Movement of pregnant cows between regions
    - Introduction of animals to the herd
    - Disease history of dams
3. **Post-mortem**

It is uncommon to see a dead newborn calf in extensive grazing conditions, so if one is found as much as possible should be done to ascertain the cause. Depending on the ambient temperature, samples from a dead calf may still produce a meaningful result if the calf or samples are moved to a cold room up to 10 hours post-time of death. If possible, the calf should be wrapped up in some sort of covering – bags, tarp, box – and put into a cold room and transported to a veterinarian or directly to a laboratory. Where possible, a veterinarian should perform the post-mortem on site. If this can't be done and where it will take longer than 4 days to get the carcass to the laboratory, the carcass should be frozen. A producer may complete a post-mortem and take photos to email to a veterinarian. Most veterinarians are probably aware of the following description but it is included for distribution to the producer if they need to do the post-mortem because the vet can't get there and it is impractical to transport the foetus.

- Body weight, crown-rump length (determines undernutrition and growth retardation)
- Time of death in relation to parturition, did it die while it was a foetus; did it die during birth or did it die as a calf and, if so, how many days old would it have been. Time of death can be ascertained by state of lungs, severed end of umbilical cord / clot, state of hooves as to whether it has walked, stomach contents as to whether it has sucked.
- Calves born alive but died of cold stress, hypoglycaemia, starvation. This can be ascertained by brown fat reserves, fat in intestinal lymphatics. Brown fat is primarily located around the kidneys and has an exceptional ability of keeping the calf warm and

providing it some energy. Brown fat production will be limited if the cow suffers nutritional stress in the last trimester.

- Birth injury or trauma can be detected by examination of ribs, liver, subcutaneous oedema, brain haemorrhage
- Role of infectious disease can be confirmed or ruled out by taking samples
- Congenital disease, deformed calf

If abortion is suspected, fresh (maybe frozen) and formalin—fixed specimens of foetal tissue (lung, heart, liver, kidney, spleen, brain) and placenta (plus cotyledons, inter-cotyledon areas – all fresh and formalin fixed samples) should be sent for laboratory examination. Examinations requested are pathological and microbiological for known pathogens. Fresh (maybe frozen) foetal abdominal contents, thoracic fluid and foetal blood should also be sent to the lab. There is a strong positive association between placental mass and foetal size at birth. Inadequate nutrition or maternal calorie undernutrition can result in in-utero growth retardation in calves.

A serum sample should be collected from the dam for serological evidence of pathogens. The veterinarian should contact the laboratory to identify whether a convalescent sample is needed. Samples from unaffected dams should also be collected.

DIAGNOSTIC CHART FOR NEONATAL CALF LOSS

If an aborted foetus and/or dead calf and/or placenta is found, package and refrigerate it and send it to the lab as soon as possible. If getting it to the lab will take longer than 4 days, then freeze it before sending it.

Post-mortem findings	Likely condition
Inspection prior to opening	
Larger calf size and or small frame cow	Stillborn or weak at birth
Carcase decomposition at birth (pre-partum death)	Abortion
Meconium (= faeces produced before birth) staining of the carcase (foetal distress during calving)	Stillborn or weak at birth
Calf not cleaned after birth (reflects maternal behaviour)	Starvation/mismothering
Not clean, e.g., mud from bogging	Mismothering
Head and shoulders swollen (subcutaneous oedema fluid)	Stillborn/Dystocia
Domed head, floppy ears, short thin hair coat, joint laxity	Abortion or premature birth
Hoof membranes present - did not stand	Stillborn or weak at birth
No hoof membranes present - the calf had walked	Starvation/mismothering/ predation
Navel cord prominent, reddish and moist	Stillborn or weak at birth
Navel cord dark, dry and shrivelled, especially if 2 to 3 days old	Starvation/mismothering
Evidence of diarrhoea - faecal material around tail or hind quarters	Dehydration
Possible congenital abnormalities (especially mouth and or perineal regions)	Weak at birth
After skin first removed	
Pale carcase (from blood loss)	Predation
Visible evidence of trauma; consistent wound punctures; missing organs AND associated haemorrhage	Predation
Tissue over the rib cage remains moist with adequate fat cover (indicating normal hydration)	Weak at birth
Tissue over the rib cage lacks lustre (indicating dehydration)	Starvation/mismothering
Swollen tongue, froth in windpipe, bruising, haemorrhages, hernias	Dystocia
Excessive red-tinged fluid in the thoracic and abdominal cavities (fibrin tags suggesting infectious agent operating)	Abortion
Lungs dark red, ‘meaty’, will not float (did not breath)	Stillborn
Lungs inflated and pink (breathed)	Weak at birth /Starvation/Mismothering
Abdominal cavity examination	
Fat reserves depleted, soft, reddened and jelly-like	Starvation/mismothering
Normal fat reserves (white; firm; around kidneys, heart and in abdominal mesenteries)	Stillborn/Weak at birth/Predation
Open the stomach	
Stomach contains clotted milk	Predation
No milk in the stomach	Weak at birth /Starvation/Mismothering

POST-MORTEM TECHNIQUE To be provided to the producer to guide post-mortem if the veterinarian cannot get to the property in a timely manner

Action	
Consider the animal's history	• Consider the site, management, handling, weather, animals around, feed, water, etc
Prepare the animal, equipment and situation for a suitable safe post-mortem	• Select a good place to do it. Make sure what is left will not affect anyone or anything adversely, e.g. the carcass can be burnt where it lies if required. This might entail shifting the animal. • If possible, do the job on freshly-dead animals. Immediately after euthanasia for very ill animals is optimum. Within hours, significant changes occur in the carcass. • Sometimes animals can carry infectious diseases so take precautions to avoid unnecessary contact with tissue and fluids. For example, do not eat and drink around the procedure, wear plastic gloves, wear shoes, have water and soap on hand to clean up, wear a mask if you think it is warranted.
Start by detailed examination of the carcass before opening	• Look over the whole animal first. Note its tag numbers, number brands, property brand, sex, age, and anything unusual. • Throughout the procedure, take careful note of what you see, and try to compare it against what you have previously seen (e.g. in killers), or what you think might be normal. If possible, have someone note and or photograph what you see.
Open and examine the carcass in a systematic way	• It is best to have cattle on their left side to do a PM for rumen weight • Skin the side from the middle of the belly and roll the hide back, along with the back and front legs which are cut through to the hip and up through under the shoulder, respectively. • Take a blood sample from the neck area. • Open the chest and gut areas without puncturing any of the organs, especially the gut. The chest is opened by slicing between each rib right to the backbone. Cut through the ribs where they bend near the sternum with either a knife, tippers, or axe. Then fold each rib back. • Have a look at the lungs, heart, liver and kidneys. Cut each out, slice it, and describe it.
At all stages, take photographs and any appropriate samples	• If you suspect a poison or something (bacteria, virus) you can grow out of these organs, put a clean sample in a sealed container, and chill it immediately. Freezing will kill most things you want to grow. And failure to chill quickly usually results in a useless sample. The best organs to sample are the liver and kidney. • Open the gut last. Sometimes opening the paunch to get an idea of what the animal has been eating can be helpful. • As soon as you get a chance, write down what you found.