How to interpret serological tests commonly used in the investigation of lower than expected reproductive performance

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Overview

This paper will focus on interpretation of the findings of tests which measure antibodies titres/levels to infectious agents which have been demonstrated to cause or be associated with lower than expected reproductive performance in beef and dairy herds. The paper will not deal with investigation of suspected vaccination failures. Further, the paper will focus on interpretation of test results for a single sampling conducted either prior to or after observed reproductive loss. Traditionally serological investigations have involved testing of paired samples collected from the same animals over some period, which is often weeks to months. However, in many cases collecting paired samples is not practically possible and veterinarians must attempt to interpret the findings from a single sampling from a herd/management group (mob).

To interpret serological test findings you need:

1. A good understanding of the immunological response of cattle to each infectious disease and what can commonly influence this response. Detectable antibodies after infection may persist for long periods and carry over from one reproductive cycle to the next or may only persist for a few months.

2. A time-line of key management events (start and finish of mating, date of pregnancy diagnosis, date(s) of weaning, date(s) of introduction or removal of any cattle (all classes) from the herd, dates when heifers/cows were vaccinated against specific diseases) in relation to when cattle were bled for serological testing. In herds/management groups which have vaccinated against specific infectious diseases it is very important that veterinarians have a thorough understanding of the vaccination protocol used by the farmer. In the authors experience too often famers have either changed the recommended timing of vaccinations to suit their mustering/management schedule e.g beef heifers vaccinated at time of selection and then again at time of pregnancy diagnosis, or employed suboptimal vaccine storage, handling and administration.

3. A good understanding of the strengths and weaknesses of each serological test. This is especially important when conducting serological testing on cattle which have been vaccinated against specific infectious diseases. For example, some serological tests e.g the AGID test for bovine viral diarrhoea virus (BVDV) or bovine pestivirus detects antibodies to mostly non-structural proteins of BVDV which are only produced when the virus replicates in cattle during natural infection or after vaccination with a modified-live vaccine. The Agar Gel Immunodiffusion (AGID) test typically does not detect antibodies produced after vaccination with the current killed vaccines. Therefore, in cattle which have been appropriately vaccinated with a killed

vaccine, subsequent detection of AGID seropositive cattle simply represents natural exposure to BVDV and not systemic infection. The BVDV Virus Neutralization Test (VNT) and Enzyme Linked Immunosorbent Antibody Assay (ELISA) generally cannot differentiate between vaccination and natural exposure to BVDV.

4. A good working relationship with the laboratory conducting the serological testing. Actively involving the diagnostic laboratory veterinarian in your investigation greatly enhances the quality of interpretation of test results.

A major problem with many/most investigations of lower than expected reproductive performance is that the investigation is typically initiated many months after the embryo-, foetal, calf-loss has occurred, and where the cause was an outbreak of infectious disease, then often the 'immunological fingerprint' is quite weak or difficult to interpret. Only a small proportion of investigations are prospective, yet these have the greatest probability of yielding genuine associations between risk factors and outcomes. Further, too often veterinarians feel pressured to provide a definitive diagnosis and simply use seropositivity as the basis for diagnosing infectious disease as the cause of the problem, when often this simply represents exposure at some point in time to the infectious agent, and is incorrectly associated with reproductive outcomes.

Both the Dairy Australia funded InCalf project and the Meat and Livestock Australia funded Cash Cow project have clearly demonstrated that nutritional, environmental and management factors are the major contributors to lower than expected reproductive performance with infectious diseases only causing significant problems in situations where an outbreak of infection coincides with critical stages of embryo, foetal or calf development. Therefore, it is very important that in any investigation that veterinarians carefully consider the likely contribution of all the known major causes of reproductive loss as well as the possible contribution of infectious diseases.

Two important contributors to misinterpretation of serological test results are:

1. Failure to bleed a representative sample of the at-risk group of cattle. Too often only cattle which have apparently experience reproductive loss are sampled rather than a cross-sectional sample of the cattle at risk in the management group. However, if a sample of cattle in the herd/group had been bled and tested prior to the period of risk of loss e.g. immediately prior to commencement of mating, then a reasonably valid interpretation of the significance of the later serological findings can be made.

2. Failure to appreciate that the risk of an outbreak of infectious disease can vary markedly between individual management groups (mobs) on the same property even though cattle may be in adjacent paddocks. Too often conclusions about contribution of specific infectious diseases to beef herd performance in particular is based on serological findings from only one or two management groups within the herd.

Investigating association between prevalence and magnitude of seropositivity and mob reproductive performance

As part of the Meat Livestock Australia funded Cash Cow project1 methodology was developed to enable causal association between the prevalence and magnitude of seropositivity for potential infectious causes of reproductive loss to be investigated. A cross-sectional sampling method was used to collect between 10 to 30 blood samples from each enrolled mob of cows at the time of either the first annual branding or weaning muster and

then again from the same females at the pregnancy diagnosis muster. Most heifer mobs were only sampled once at the pregnancy diagnosis muster. Also, at the pregnancy diagnosis muster vaginal mucus samples were collected from the same females selected for blood sampling. The following sampling guideline was used:

- For mobs of < 100 heifers/cows: 10-15 samples i.e., every 10th female, regardless of pregnancy or lactation status, presenting at the crush was sampled
- For mobs of 100 - 200 heifers/cows 15-20 samples were collected
- For mobs of 200 - 300 heifers/cows 20-30 samples were collected
- For mobs of > 300 heifers/cows 30 samples were collected.

The number of females sampled from each mob was based on recommendations provided by Dr John Morton (Jemora Pty Ltd) with the 90% confidence intervals for various numbers of animals tested and for the various percentages that test negative provided in Table 1.

Table 1. Determining the precision of the serological profile with different numbers of animals tested.

% of animals tested that were seronegative	Likely % of animals in herd/group that are seronegative		
	10 animals sampled	20 animals sampled	30 animals sampled
0 (i.e., all tested positive)	0-26	0-14	0-10
30	9-61	14-51	17-47
50	22-78	30-70	34-66
70	39-91	49-86	53-83
100 (i.e., all tested negative)	74-100	86-100	90-100

Testing to determine the association between vibriosis and reproductive performance

Vibriosis is a venereal disease caused by infection with C. fetus subsp. venerealis. It can cause transient infertility, early embryonic death, and sporadic abortion in cattle. Typically, the earliest indication that an outbreak of vibriosis has occurred in a mob is the finding of a lower than expected pregnancy rate. However, in the Cash Cow project a high prevalence of infection in a mob at the time of pregnancy diagnosis was associated with higher calf wastage i.e.losses between confirmed pregnancy and weaning. Currently, vaginal mucus samples are tested using an IgA ELISA2 which has a reported specificity of 98.5%. ELISA results are reported as positive, inconclusive or suspect (low positive result), and negative. The prevalence of positive reactors provides a useful estimate of the prevalence of C. fetus subsp. venerealis infection, which in turn provides an estimate of the risk of vibriosis affecting mob performance. The suggested mob prevalence risk categories are Nil: 0%;

Moderate: >0 to <30%, and High: ⩾30% ELISA test positive. Veterinarians need to apply some caution when interpreting the significance of a finding of a high prevalence of seropositivity as there is some evidence2 that titres can persist for quite long period and may carry over from one breeding season to the next. A high prevalence could represent exposure prior to mating or expected peak period of mating. Equally in endemically infected mobs it could represent both infections which have occurred during the current mating period and those that occurred in the immediate past mating period, with the latter females likely to be immune and thus unaffected by the current exposure. When high prevalences of seropositivity are detected this should trigger investigation of the presence of infection in bulls mated to these females and probably bulls in adjacent paddocks. The magnitude of the ELISA test result for seropositive animals does not indicate whether infection is recent.

Testing to determine the association between bovine pestivirus infection and reproductive performance

BVDV infection of naïve cattle around the time of mating and during the first four to five months of pregnancy can result in a wide range of losses, including lower than expected pregnancy rates and/or weaning rates and reduced turn-off of young cattle. ELISA, VNT and AGID tests3 can be used to determine the seroprevalence of infection in unvaccinated mobs of cattle. The latter can also be used to estimate exposure to BVDV in vaccinated mobs. The suggested mob seroprevalence risk categories are Low: <20%, Moderate: 20-80%, and High: >80% seropositive.

The AGID test can also be used to determine whether infection is recent4. Cattle with an AGID test results of ⩾3 – seropositive are likely to have been infected/exposed to BVDV in the past 1 - 9months. Suggested mob risk categories of recent BVDV infection are Low: <10%, Moderate: 10-30%, and High: >30% AGID test results ⩾3. All animals from a mob were assigned the same risk category based on the serological findings for the sample of animals tested.

Testing to determine the association between leptospirosis and reproductive performance

Leptospirosis has been associated with abortions, stillbirths, and birth of weak calves in dairy and beef cattle herds in Australia. However, there is increasing evidence that the cattle adapted serovar Leptospira borgpetersenii serovar hardo type Hardjobovis (L. hardjo) is now primarily a 'parasite' of the kidney of infected cattle presenting primarily a risk to people working with these cattle, rather than being a significant cause of reproductive loss. L. hardjo) and Leptospira interrogans serovar pomona (L. pomona). Typically diagnostic laboratories will only routinely test for antibodies to L. hardjo and L. pomona using the Microscopic Agglutination Test (MAT). Samples with a MAT titre of >100 for each serovar are considered positive and samples with an MAT of ⩾800 are considered indicative of recent infection5.

Vaccination of cattle with commercially available leptospirosis vaccines does induce MAT titres of ⩾200 for periods of approximately six months after vaccination, and thus great caution should be applied when interpreting serological results for mobs which have a history of leptospirosis vaccination. Further, it is well recognised that following natural infection MAT titres rise and fall quite quickly (often within several months), with abortions often occurring when titres have decreased to low or undetectable values. Thus, the interpretation of MAT titres of ⩽400 can be problematic as they may be associated with recent infection or they may simply indicate past exposure. MAT titres of ⩾800 generally

indicate that infection is relatively recent in unvaccinated herds, and thus enable a more direct association between reproductive loss and infection to be investigated. The suggested mob prevalence of recent infection (i.e. MAT titres ≥800) risk categories are Low: <10%, Moderate: 10-30%, and High: >30%.

Testing to determine the association between Neospora caninum infection and reproductive performance

Neosporosis is recognised worldwide as one of the most important infectious causes of abortion in cattle . The primary sources of Neospora caninum are wild and domestic dogs and other canids, such as foxes and dingoes. Abortions due to N.caninum have been reported in both dairy and beef cattle in Australia. The ELISA used in the Cash Cow project was an indirect ELISA6 developed at the Elizabeth Macarthur Agricultural Institute. This ELISA has been validated against panels of sera tested by both indirect fluorescent antibody technique and western blotting, and has been shown to have a sensitivity of 100% and specificity of 99% (Kirkland pers com). Suggested mob seroprevalence risk categories are Nil: 0%, Low: 0-20%, Moderate to High: ≥20%.

Testing to determine the association between Bovine Ephemeral Fever (BEF) virus infection and reproductive performance

BEF virus infections occur commonly in cattle across northern Australia, particularly during the summer-autumn period. Infection of naïve pregnant females has been reported to result in increased rates of abortion. A VNT7 is used to test for antibodies to BEF virus. Samples with a VNT titre of >40 are considered positive and those with a titre of >640 indicative of recent infection (Kirkland pers com). Suggested mob seroprevalence risk categories are Low: <20%, Moderate: 20-80%, and High: >80% seropositive. The suggested mob prevalence of recent infection (i.e. BEF VNT titres ≥640) risk categories are Low: <10%, Moderate: 10-30%, and High: >30%.

Conclusions

It is clear that veterinarians should take a conservative approach when interpreting serological test results, and be wary of misinterpreting evidence of exposure to an infectious disease as evidence for cause of observed reproductive loss. Perhaps in herds that do not vaccinate against specific infectious causes of reproductive loss, the approach veterinarians could take is to routinely collect blood from a representative sample of cattle in each management group at the time of pregnancy diagnosis. Sera from these samplings would be stored frozen, and could be subsequently tested as part of an investigation of reproductive loss, or discarded prior to the next round of annual pregnancy diagnosis of the herd. This approach is similar to that recommended for investigating and managing clinical mastitis in dairy herds. The major reason for recommending this is that it enables the veterinarian to make a scientifically reasonable association between the findings of the serological testing and the observed reproductive outcome.

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