PRACTICAL APPROACH TO INFECTIOUS BOVINE ABORTION DIAGNOSIS

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1. INTRODUCTION

Bovine abortion is an important cause of economic loss to the cattle livestock industry. In California this may as much as $700 for each cow that has aborted (Thurmond & Picanso, 1990). Kossaibati & Esslemont (1997) estimated the average cost of calf mortality in English dairy herds was £310 per calf death at 1995 prices; for culling an additional cow following illness, such as abortion, it was £770 per cow culled.

The abortion incidence rate varies widely, depending on the health status of individual herds. In one New Zealand study, 61% of dairy herds had an abortion rate of ≤ 5% whilst 16% experienced a rate of ≥ 10% that was described as epidemic (Thobokwe & Heuer, 2004). In a study of reproductive performance in 66 Canadian beef herds the risk of abortion occurring was 1.5% (range 0-7.3%) and the actual abortion rate was 2.7% (Waldner, 2005). Where there is a high risk of abortion associated with a specific disease, for example endemic neosporosis, the abortion rate over a short period of a few months may be as high as 33% of all pregnancies (Dubey, 2005).

In England and Wales, there was 5662 abortion episodes reported and investigated fully by the Veterinary Laboratories Agency (VLA) during 2003 (Anon., 2005). Some of these were included in the 6872 cows that were examined for evidence of brucellosis that year, four of which were confirmed positive for *B. abortus* infection. Table I summarises the most common diagnoses for fetopathy within this period. The positive diagnostic rate was only 17%.

Table I. **Infectious agents most commonly identified and associated with 5662 bovine abortion episodes investigated in the UK during 2003**

	Abortion episodes	
Infectious agent identified	***Number***	***%***
Neospora caninum	367	6
Bacillus licheniformis	145	3
Salmonella spp	131	2
Arcanobacterium pyogenes	90	1.6
All fungi	54	< 1

Bovine viral diarrhoea virus	50	< 1
Other infectious agents	85	1.4
Other diagnoses	126	2
No diagnosis made	4614	83

These differ generally from results obtained from the eight Scottish Agricultural College (SAC) Veterinary Disease Surveillance Centres in the years 2000 and 2001, who found that of 739 abortion episodes investigated 6% were associated with bovine viral diarrhoea virus (BVDv) infection (Crawshaw *et al.* 2003). During the period 2000-2005, 2681 aborted bovine fetuses were examined for evidence of *N. caninum* infection and only 1.9% met the diagnostic criteria for a positive diagnosis to be made (Caldow & Somerville 2005).

These differences may be explained in several ways. The diagnostic criteria may vary from one diagnostic laboratory to another. For example, the criteria for positive diagnosis of BVDv by the SAC include recognition of related gross or histological lesions, or presence of virus specific antigen or antibody in the aborted fetus (Crawshaw *et al.* 2003); the VLA have developed a polymerase chain reaction method to detect viral antigen in bovine fetuses as their preferred diagnostic tool (Anon., 2005). The interpretation of laboratory results may vary. For example, the diagnostic criteria for a fetopathy associated with *N. caninum* could be demonstration of microscopic lesions in the brain or myocardium of the fetus together with a seropositive fetus or dam. Natural infection with this disease is by vertical transmission from infected dam to the fetus and congenital infection of a live born fetus is common. Therefore, it does not mean necessarily that pathognomic lesions present in a fetus aborted at 32 weeks gestation signify neosporosis was the direct cause of abortion; most associated pregnancy losses occur around 20-24 weeks gestation (Dubey, 2005).

Of the six named micro-organisms listed in Table I, it is possible to vaccinate against only two, *Salmonella* sp. and BVDv. Included under the heading “Other infectious organisms” in Table I are *Leptospira hardjo* and bovine herpesvirus-1, infections that can also be controlled by vaccination. If a purely pragmatic approach for reducing bovine abortion incidence on farms was to be devised, based only on vaccination, the diagnostic output of abortion investigations could be based solely on determining whether any of those four named infections were implicated. Such an approach should be cost effective for the farmer.

2. HERD HEALTH STATUS AND ZOONOTIC CONSIDERATIONS RELATED TO BOVINE ABORTION PATHOGENS

A workshop on bovine abortion diagnosis and its control was held in Edinburgh at the World Buiatrics Conference 1995. One of its outcomes was to identify the context within which an abortion investigation should be conducted, as far as overall herd health or public health issues were concerned. If this approach was taken, the number of microorganisms screened for within such an investigation could be reduced. The herd health and zoonotic issues that should be considered are set out below:

2.1 Isolation of specific abortion pathogens that also affect adversely general herd health status and productivity

Abortion could be considered as just one manifestation of a broader disease pattern on the farm. For example, on a farm where Mucosal Disease due to BVDv infection is diagnosed frequently in juvenile cattle, lowered fertility and abortion in cattle may be an additional cause of economic loss on that unit. The extent to which BVDv associated abortions contributed to that economic loss would be an important piece of information when deciding the cost benefit of an effective vaccination programme. By contrast, there is little to be gained by identifying β-haemolytic

Streptococci in fetal stomach contents; it is a common commensal in the mucosa of the upper respiratory and lower urinogenital tracts of cattle and does not survive for long outside the animal host. It is an opportunistic pathogen and of little importance as far as general herd health and productivity issues are concerned.

2.2 Cost effectiveness of identifying abortion pathogens

The ease and cost of isolating and identifying primary pathogens associated with both abortion and poor herd health status varies greatly. Bovine herpesvirus-1 infection can be associated with both upper respiratory tract infections in young cattle, and pustular vulvo-vaginitis and abortion in naïve breeding cattle although it is uncommon to find either clinical syndrome occurring concurrently within a herd. Laboratory diagnosis is straight forward for all three diseases, based on antigen detection using immunofluorescence: in a case of abortion, antigen can be detected similarly in fetal kidney and adrenal gland (Kirkpatrick, 1990). This diagnostic method is reliable and not expensive, costing £9.40 in the U.K. In contrast, consider the cost effectiveness of a full abortion enquiry involving screening fetal stomach contents for possible *Campylobacter fetus* subsp. in a herd where artificial insemination (AI) is used commonly to breed cows and heifers. Direct culture onto blood agar as well as a selective medium, modified Skirrow's, and incubation under microaerophilic conditions for 72 hours is relatively costly; the sensitivity of this method is approximately 80% (Lander, 1990). The basic cost of this in the U.K is around £27.00. Whilst this cost is justified in a beef suckler herd or a group of pregnant dairy heifers where abortion was a problem and these cattle were bred using natural service, it is not cost effective to eliminate the possibility of C. *fetus* subsp being involved in sporadic abortions in a dairy herd bred mostly using AI.

2.3 Zoonotic diseases associated with abortion pathogens

Bovine brucellosis has been designated by the World Organisation for Animal Health (OIE) as a List B disease of public health importance. Most countries screen cattle routinely for evidence of *Brucella abortus* infection. Screening for the presence of *B. abortus* antibodies is usually carried out in dairy cows using bulk milk; in beef herds, serum is obtained every 12 or 24 months from all breeding cattle for the same purpose. Specific investigations to eliminate this organism as a cause of sporadic bovine abortion episodes are commonplace. For example, in Canada during 2002, 9% of all cattle testing for brucellosis was within the context of specific disease investigations such as for abortion (Anon., 2003). Diagnosis was based primarily on specific antibody detection in maternal milk or serum. Effective disease control is based on slaughter of seropositive cattle following natural exposure and infection. Vaccination of young cattle with S19 vaccine may limit the cycle of natural infection within endemically infected herds.

Leptospirosis is classified as a List B disease in multiple species, for similar reasons. However, its diagnosis and control varies worldwide. In the OIE Report of 2002, only two countries reported on this disease. This may reflect the low priority for its control in the majority of countries or, in those countries with intensive livestock farming enterprises, the success of an effective cattle vaccination policy that has impacted favourably on human infection incidence. In the Zoonosis Report, United Kingdom, 2000 (the last year where information was presented within the public domain) of 54 confirmed human cases of leptospirosis only 12 were attributable to *Leptospirosis interrogans* var *hardjo*. More recently, Jansen *et al.* (2005) investigated 102 laboratory-confirmed cases of leptospirosis in the German population during 1997-2000 and only one was attributed to *L. interrogans* var *hardjo* infection.

None the less, lowered fertility and abortion in cattle associate with *L. interrogans* var *hardjo* infection is still an important cause of economic loss to farmers (Guitian *et al.* 1999).

Another zoonotic disease is salmonellosis. In northern mainland Europe the origin of approximately 15% of human subgroup 1 *Salmonella* infections originates from pig meat. In cattle, subgroup 1 *Salmonella* infections are associated with both enteric disease and septicaemia in calves, and acute haemorrhagic diarrhoea in newly calved heifers and cows. *Salmonella* sp. are opportunistic pathogens isolated from aborted lambs and calves. In the UK, salmonellosis is a Reportable Disease and veterinary investigation staff visit farms where a positive diagnosis has been made. Diagnosis is straight forward; fetal stomach contents are inoculated into enrichment media such as selenite broth and then on selective media such as brilliant green (BG), or else plated directly on BG agar (Quinn *et al.* 2002).

2.4 Availability of effective control measures for specific infectious diseases associate with abortion

Vaccination against infectious disease is considered by many to be the greatest public health achievement of the 20th century. In veterinary medicine the most successful vaccines are toxoids and those directed at viral surface proteins (Pier, 2004). One of the main purposes for identifying the aetiological agent associated with any infectious disease is to devise effective control measures against it. As far as infectious bovine abortion is concerned, vaccination against specific causal pathogens is probably the most cost effective control method. Of the microbiological agents listed in Table I vaccines are available against only two pathogens, BVDv and *Salmonella* sp. Vaccines have been developed against only three of the other 32 microorganisms that have been isolated from aborted bovine fetuses, bHV-1, *B. abortus* and *L. hardjo*. Thus, abortion investigations could be focused solely on determining whether the cause of abortion was associated with any of those five pathogens which are amenable to control in this way.

Another method of disease control is by compulsory slaughter of infected cattle; for example, *B. abortus* infection in countries with intensive dairy farming systems. In other countries, vaccination is preferred. In Scandinavia, BVDv infection is being eradicated by identification of persistently infected (PI) cattle and culling; a similar policy has been implemented in Austria.

Neosporosis is another infection associated with bovine abortion where culling has been advocated as the preferred control measure. Such action might be justified in herds where the seroprevalence to infection is low at around 5-10%, but not as the sole control measure. This approach is totally impractical where infection is endemic and the seroprevalence is greater than 50%, as is the case in a number of dairy herds in the UK (Murray, 2004).

Other simple control measures could be introduced into a herd if known specific infections are identified. For example, abortion occurring in a beef herd where hired bulls are used to breed cows and heifers can be minimised by devising alternative breeding strategies. Hired bulls are responsible for the introduction and dissemination of BVDv, b-HV1, *L. hardjo* and *C. fetus* subsp. *venerialis* in to susceptible herds when they are moved from farm to farm as part of normal cattle breeding practice (McGowan & Murray, 1999).

2.5 Suitability of serological methods for identifying abortion pathogens

Autolysis is a common characteristic of aborted fetal tissues. Such tissue is unsuitable for reliable detection of possible bacterial or viral antigen associated with bovine infectious abortion using routine veterinary laboratory diagnostic techniques available currently. Often there is a long time period, weeks or even months, between the initial microbiological challenge of the pregnant dam and her fetus and the abortion resulting from that infection. Direct evidence of the involvement of a primary abortifacient agent, associated with such an abortion, has disappeared long ago. Therefore,

the pathologist must rely on indirect methods in an attempt to identify possible infectious causes of abortion. Serology, utilising both fetal fluids and maternal serum, is one such tool.

The use of bulk milk antibody titres against *B. abortus*, BVDv, b-HV1, *L. hardjo* and *N. caninum* has allowed the diagnostician to mass screen dairy cattle and determine the herd disease status with regard to these infections. Case finding, or screening, individual cows that have aborted may be carried out by comparing their individual serological profile for these infections against the remainder of the herd that has remained healthy. Bulk milk antibodies, used in this way, give an indication of the weight of active infection within the herd. By comparing the serological profile of a cow that has aborted with that of the whole herd the predictive value of serology as a diagnostic test to identify probable primary infective abortifacient agents involved in an abortion outbreak is increased. The use of this technique is described below for investigating of early fetal loss in pregnant dairy cattle.

3. INVESTIGATION OF EARLY FETAL LOSS IN PREGNANT DAIRY CATTLE

During 2003-2005, eleven farm animal veterinary practices located in Northern Ireland and the west of England provided routine weekly fertility visits to their dairy clients. This service included manual pregnancy diagnosis 35-49 days after service confirmed using ultrasound, diagnosis of cystic ovarian disease and treatment, and assistance with heat detection. In 33 of these herds, the stockmen complained that cows were returning to service around 49-80 days gestation after being confirmed earlier as pregnant. There was no visual evidence of abortion presented by any of the 117 cows affected. Subsequently, these cows were confirmed as non-pregnant and cycling using ultrasound and were rebred when next in heat.

Vaccination was carried out to control *L. hardjo* infection in 21 herds, for BVDv in 10 herds, and against b-HV1 infection in three.

Bulk milk samples were taken from each herd immediately after this problem was discussed first with their veterinary surgeon. Subsequently, serum samples were obtained from each pregnant cow that aborted less than 80 days gestation; the first when the cow was first diagnosed as not pregnant, the second at least 28 days later. All samples were identified by farm of origin, date, and herd milk recording number. They were stored at -20°C and submitted to the Veterinary Laboratories Agency diagnostic unit at Barton Hall, Preston. Bulk milk antibody titres were measured for BVDv, b-HV1, and *L. hardjo* using enzyme linked immunoassay (ELISA); from the results, given in optical density (OD) units, the herd infectious disease status or category was estimated. In two herds not vaccinating against BVDv, there was no evidence of viral challenge and infection. There was no evidence of b-HV1 infection in a further 15 unvaccinated herds. In 5 herds not vaccinating against *L.* hardjo there was no evidence of natural exposure to infection.

Antibodies against BVDv, b-HV1, and *N. caninum* in cows' paired sera were measured using ELISA and results given in OD units or percentage positivity for N. *caninum*; the microscopic agglutination test (MAT) was used to estimate *L. hardjo* antibody titres, the lowest dilution being 1/50.

3.1 Cow seroprevalence against known pathogens within context of herd infectious disease status

3.1.1 Bovine viral diarrhoea virus

Table II describes the herd disease category for the 10 vaccinated and 23 unvaccinated herds investigated, and the number of cows always seronegative for this infection within herds of each category. Cows with OD units of < 0.2 were considered to be seronegative.

Negative or low positive category herds were probably naïve and susceptible to new sources of infection entering the herd through purchasing cows or bulls of unknown BVDv infection status. Such herds contained mostly seronegative healthy cattle with little virus present to challenge naïve cattle. The interpretation of serology results for cows always seronegative in these herds was that they had not been challenged by the virus.

The 10 herds who routinely vaccinated cows fell into the moderate or high infection status categories where, in the absence of control measures being focused on identification and culling of PI cattle together with vaccination, it was expected that PI cattle were present in these herds. Of 16 unvaccinated herds in the same categories, it was assumed that acute active infection was present. Every cow sampled in these herds, except the 23 seronegative cows, had BVDv titres of > 0.3 OD units. Therefore, cows that were always seronegative were considered to be potential PI cattle themselves.

Table II. **Numbers of dairy herds categorised for bovine viral diarrhoea virus disease status, and related cows which had aborted and were always seronegative for the virus**

	Vaccinated herds		**Unvaccinated herds**	
Herd disease category	***Number***	***Number of cows seronegative***	***Number***	***Number of cows seronegative***
Negative (OD units < 0.2)	0	0	2	2
Low positive (OD units 0.2-0.4)	0	0	5	5
Moderate positive (OD units > 0.4-0.7)	2	1	8	4
High positive (OD units > 0.7)	8	7	8	11

Differences between the actual and expected numbers comprising Table II were investigated using goodness of fit analysis; the chi-square value was 9.57 (p 0.01).

In two herds categorised as high positive with bulk milk titres of 0.72 and 0.86, the BVDv antibody titres of two cows which had aborted were > 0.90 OD units. These results are consistent with cows aborting following natural infection.

It was concluded that 25 (21%) of all 117 cows that presented with early fetal loss may be associated with BVDv infection, based solely on serological investigations.

3.1.2 Bovine herpesvirus-1

Table III describes the disease category for the herds investigated. No cows had significant rising titres or titres consistently > 0.9 OD units after loss of their pregnancy.

It was concluded that bHV-1 infection was not associated with early fetal losses.

Table III: **Numbers of dairy herds categorised for bovine herpesvirus-1 infection status, and related cows which had aborted with significant rising titres**

	Vaccinated herds		Unvaccinated herds	
Herd disease category	***Number***	***Number of cows with significant rising titres***	***Number***	***Number of cows with significant rising titre***
Negative (OD units < 0.2)	0	0	16	0
Low positive (OD units 0.2-0.4)	0	0	6	0
Moderate positive (OD units > 0.4-0.7)	0	0	6	0
High positive (OD units > 0.7)	3	0	2	0

3.1.3 Leptospira hardjo

Table IV describes the herd disease category for the 21 vaccinated and 12 unvaccinated herds investigated. Fetal losses occurred in two vaccinated herds; the three cows affected presented with microscopic agglutination titres (MATs) of 1/400, rather high for vaccinated cattle. In unvaccinated herds, the MATs were ≥ 1/400 for all 11cows that aborted in three high positive herds where acute active infection was present; in one of these herds, four cows were also seronegative for BVDv infection.

Table IV: **Numbers of dairy herds categorised for *Leptospira hardjo* infection status, and related cows which had aborted with MATs ≥ 1/200**

	Vaccinated herds		Unvaccinated herds	
Herd disease category	***Number***	***Number of cows with MATs ≥ 1/200***	***Number***	***Number of cows with MATs ≥ 1/200***
Negative (OD units < 0.2)	1	0	4	0
Low positive (OD units 0.2-0.4)	2	0	1	0
Moderate positive (OD units > 0.4-0.7)	5	0	0	0
High positive (OD units > 0.7)	13	3	7	11

It was concluded that acute *L. hardjo* infection might be responsible for 11 (9%) of the early fetal deaths, possibly involving concurrent BVDv infection in a third of these.

3.1.4 Neospora caninum

At the time of writing, it was not possible to carry out bulk milk antibody estimations for *N. caninum* infection. There was evidence of infection present in cattle on 15 (45%) of the 33 herds examined, cows having ELISA percentage positivity (pp) titres of ≥ 20. Of the 117 cows examined, 21 (18%) were seropositive; 7 presented with titres ≥ 60 pp and 8 with titres 40-60 percent positivity. There were three cows that were always seronegative for BVDv that presented with titres ≥ 60 pp. Examination of serum from aborting cattle is indicative of prior exposure to the organism and no definitive antibody value should be considered diagnostic for neosporosis (Dubey 2005). However, for early fetal death to be associated with this infection only pp values ≥ 60 were considered significant. Canada *et al.* (2004) have shown that lower positive titres give 100%

sensitivity but lower specificity for serodiagnosis of *N. caninum* associated abortion, especially if other infectious microorganisms are considered as well.

It was concluded that 15 (13%) of the 117 cows examined could have suffered early fetal losses associated with *N. caninum* infection, a proportion similar to that described by Davison, Otter and Trees (1999) in UK cattle.

4. SUMMARY

The diagnosis of bovine abortion is one of the most difficult problems confronting the veterinary practitioner in general practice. With the eradication of bovine brucellosis in the United Kingdom and many other European countries, relatively few bovine abortion episodes are investigated fully. Of 6868 cows sampled for evidence of *Brucella abortus* infection in the United Kingdom during 2003, four breeding cows were confirmed positive. From a breeding cattle population of approximately 2.5 million the Veterinary Laboratories Agency investigated 5662 abortion episodes and achieved a diagnostic rate of 17%. There are over 30 potential pathogens that have been associated with abortion; only four related infections can be controlled by herd vaccination and another by compulsory slaughter. There is no economic advantage to the farmer for a full investigation of every abortion episode, except where abortion is only one manifestation of a wider disease pattern associated with a specific pathogen or risk factor on a farm; for example, endemic bovine viral diarrhoea virus (BVDv) infection. Justification for this opinion will be presented and illustrated by results of an investigation into early fetal death occurring in 33 dairy herds, involving 117 cows that aborted between 49-80 days gestation. A herd diagnostic approach is described, comparing bulk milk antibody titres related to the herd infectious disease status with paired maternal serological profiles of aborting cows. It was concluded that 25% of early fetal losses could be associated with BVDv infection.

5. KEY WORDS

Bovine abortion, diagnosis, BVD.

6. RESUME

Le diagnostic des avortements bovins est un des problèmes les plus délicats auxquels sont confrontés les vétérinaires en pratique courante. Depuis l'éradication de la brucellose bovine dans le Royaume-Uni et dans de nombreux pays européens, peu d'investigations complètes ont été réalisées lors d'avortements en série. Sur 6868 vaches testées vis-à-vis d'une infection à *Brucella abortus* au Royaume-Uni en 2003, quatre vaches reproductrices ont été confirmées positives. Sur une population de bovins reproducteurs d'environ 2,5 millions, le Veterinary Laboratories Agency a analysé 5662 épisodes d'avortement pour lesquels un diagnostic a été possible dans 17 % des cas. Il existe actuellement plus de 30 pathogènes potentiellement abortifs. Le contrôle de 4 de ces agents pathogènes est possible par la vaccination, l'abattage obligatoire permettant d'éliminer un cinquième pathogène. Actuellement, il n'y a pas d'avantage économique pour l'éleveur à réaliser une investigation complète et systématique lors d'avortements, sauf si l'avortement est une des manifestations d'une maladie plus générale provoquée par un pathogène spécifique ou associé à un facteur de risque particulier dans l'élevage (par exemple l'infection par le virus de la diarrhée virale-maladies des muqueuses -BVDv-). Cet argument sera discuté et justifié dans cet exposé par la présentation de résultats provenant d'une étude diagnostique sur des cas de mortalité foetale précoce dans 33 élevages laitiers, impliquant 117 vaches qui ont avorté entre 49 et 80 jours de gestation. Une approche diagnostique au niveau du troupeau est décrite en comparant les titres en anticorps dans les laits de tank, qui corrèlent au statut infectieux de l'élevage, avec les profils de

sérologie couplée chez les vaches ayant avorté. Les résultats montrent que 25 % des pertes foetales précoces peuvent être associés à une infection par le BVDv.

7. MOTS CLES

Avortements bovins, diagnostic, BVD.

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