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AETIOLOGY OF BOVINE ABORTIONS IN IRELAND
TETIOLOGI OI DOVINE TROMITONS IN INCLEMEN
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1. Introduction:

Abortion in bovine animals is a predominant occurrence throughout Ireland. There is a vast range of causative agents of abortion of infectious and non-infectious origin. The aim of the present work was the collection and analysis of data concerning bovine abortions caused by infective agents in Ireland between 2006 and 2011. Abortion in cattle is defined as the loss of the foetus before its development is sufficiently advanced to allow survival (McGavin et al., 2001). Early embryonic mortality occurs up to day 42 of the conception while stillbirth is defined as the delivery of a dead foetus at a stage of development when it should have been viable (McGavin et al., 2001). Pathological processes that can occur during a pregnancy can be as a consequence of an endogenous or an exogenous cause. Exogenous causes include physical causes (such as extreme temperature, irradiation or too little amniotic fluid), dietetic problems, nutritional deficiencies or due to infections. Pathological processes can result in the termination of a pregnancy during many different stages of the pregnancy. Different abortifacients cause the death of the foetus at different stages during the pregnancy.

There is a range of pathophysiological processes which can progress resulting in the destruction of the foetus in the uterus including resorption, foetal mummification, maceration and emphysema may develop. Autolysis of the aborted foetus is also commonly observed (Maxie, 2007). Adventitial placentation may also develop. This pathological process is as a result of inflammatory destruction of the endometrium preventing the progression of the pregnancy and so terminating as an abortion (Maxie, 2007). In addition, the cotyledonary placenta type of ruminants provides a large potential area for the accumulation of exudate and infectious agents further exposing the risk of bovine abortions (McGavin and Zachary, 2007). The pregnant uterus is much more sensitive to pathogens in comparison to the immature or inactive one. There are various routes of infection which can reach the foetus such as a hematogenous way, via the lymph system, a transplacental infection or an ascending infection.

On average each year cattle farms experience abortion cases. Abortions can occur sporadically as isolated cases but also abortion storms may evolve in a herd. If more than 3 % of a herd abort in a single year this becomes an issue of major concern for the herd owner, an examination of the herd's health status should be done and the causative pathogen identified (Department of Agriculture, Food and the Marine and Agri-Food Biosciences Institute, 2011). The causing pathogen may spread to other cattle in the herd or it may lie dormant in the herd

becoming endemic. Consequently farms experience a substantial loss as a result. If an abortion storm erupts on a farm this can have very serious financial implications for the farm and its future viability. For instance, the economic loss due to an outbreak of bovine viral diarrhoea (BVD) in a herd is an issue of huge concern. In 2004 in the UK it was calculated that in a 100 cow beef herd the losses can exceed £45,000 over a 10 year period while losses in the dairy industry are estimated at twice this level with reproductive losses being the most significant (Cattle Health Certification Standards, 2012). The cost of a single abortion can be difficult to calculate precisely but an estimate of £630/€700 can be made for a dairy herd (Cabell, 2007).

The Agri-Food Biosciences Institute (AFBI) provides veterinary laboratory services to the state and private veterinary practitioners in Northern Ireland (Figure 1.). The Department of Agriculture, Food and the Marine (DAFM) of the Irish Government provide veterinary laboratory services through the regulation of the Central Veterinary Research Laboratory located at Backweston campus, Celbridge, County Kildare and through routine diagnostic procedures provided at six Regional Veterinary Laboratories (RVL's) located in Athlone, Cork, Dublin, Kilkenny, Limerick and Sligo (Figure 2.) (Department of Agriculture, Food and the Marine and Agri-Food Biosciences Institute, 2012). The role of the veterinary laboratory services is to implement Department of Agriculture and Food policy with respect to animal health and welfare and veterinary public health. The six Regional Veterinary Laboratories act as multidisciplinary laboratories examining samples received from private veterinary practitioners. The role of these laboratories is to provide diagnostic tests enabling the referring veterinarian to implement efficient treatment and control measures if necessary. All bovine abortions in Ireland must be notified to the veterinary profession and aborted foetuses and placentas are then submitted to the laboratory services for investigation and diagnosis. Blood samples from aborted cows have also been submitted and help contribute to the final diagnosis. Often the diagnostic rate for abortion in cattle is quite low depending on the preservation of the carcass and/or the samples submitted. In cattle a delay is often observed between foetal death and expulsion and so the foetus undergoes advanced autolysis before being examined therefore inhibiting the sensitivity of the diagnostic tests. As a result it is advised to submit the samples as soon as possible and with the appropriate preservative methods (Department of Agriculture, Food and the Marine and Agri-Food Biosciences Institute, 2011). There are a wide range of causative agents of abortion in cattle. Unfortunately many of the agents causing abortions are never diagnosed and left unexplained.

The aim of the present work was evaluation of the frequency and aetiology of bovine abortions caused by infective agents.

2. Materials and Methods:

During the past two years the Department of Agriculture, Food and the Marine (DAFM) veterinary laboratories in Ireland and the Agri-Food and Biosciences Institute (AFBI) laboratories of Northern Ireland have compiled the All-island Animal Disease Surveillance Reports for 2010 and 2011. Additionally, the Regional Veterinary Laboratories (RVL's) in Ireland prepared surveillance reports from the years 2006 to 2009. In each of these reports a section was dedicated to bovine abortion discussing the findings of the laboratories, the methods of detection used and the comparison of findings from previous years.

If the submission of the entire foetus to the laboratory is not possible the RVL's advise the veterinary practitioners to submit as many of the following tissues as possible: stomach fluid, pleural fluid, a section of brain tissue/placenta/thyroid gland/thymus/spleen or a blood sample from the dam of the aborted foetus as well as from any other cows that aborted or have been proven to be non-pregnant (Department of Agriculture, Food and the Marine and Agri-Food Biosciences Institute, 2011). The correct packaging and appropriate preservation of the samples submitted is important to ensure the correct handling and swift delivery of the samples. The packaging must follow the EU guidelines of the European Agreement for Transportation of Dangerous Goods regulation 2007 (ADR 2007) whereby the material must be placed in a leak-proof primary container which then must be placed in a leak-proof secondary container and a material capable of absorbing the content of the primary container must be placed in between and "Biological substances, Category B" must be clearly printed on the outside of the outer box or padded envelope. All specimens to be examined must be packaged in accordance with the classification of different pathogens in risk group categories classified by the World Health Organisation. A submission form must accompany the sample sent to the laboratory including all the information about the sample and the required tests to be carried out (Department of Agriculture, Food and the Marine laboratory services, 2012). Abortions are notifiable diseases and all cases should be reported to the Department of Agriculture. A blood sample must be submitted to the specifically equipped brucellosis laboratory in Cork and the foetus and placenta should be sent to one of the Regional Veterinary Laboratories for examination.

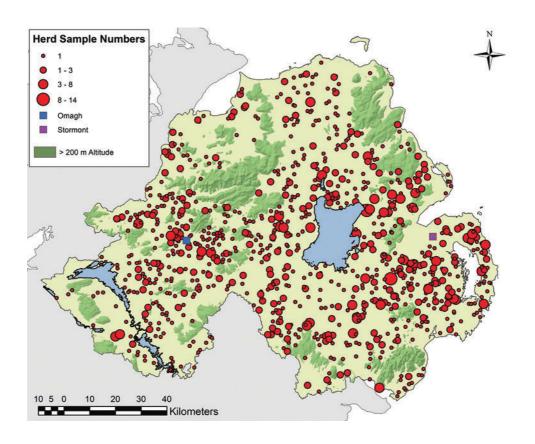
On arrival of the specimen to be examined at the laboratories there are various diagnostic methods that can be used. The examination of the foetal carcass is central to identifying the causative agent and preferably accompanied with the foetal membranes. The following methods are employed in veterinary laboratories throughout Ireland to help achieve a diagnosis:

- Gross lesions on the placenta and/or foetus can help establish a diagnosis of mycotic abortion. Necrotizing placentitis is observed in the case of Aspergillus spp.
- Placentitis is evident in the cases of *Arcanobacterium (Trueperella) pyogenes* as a result of bacteraemia in the dam leading to placentitis and foetal autolysis.
- Contents of foetal fluids are routinely examined in particular the stomach contents are collected from all foetuses. Serological tests are also performed on foetal thoracic fluid which is considered to be an acceptable analogue of foetal serum.
- Blood from the heart or pleural fluid is collected for serological examination.
 Depending on the degree of autolysis of the foetus, tissues can be taken for microbiological or histopathological examination additionally.
- To detect *Leptospira interogens* serovar hardjo specific antibodies the thoracic fluid should be collected from the foetus and examined using solid phase immunoassay method. Enzyme Linked Immunosorbent Assay (ELISA), Polymerase Chain Reaction (PCR), Microscopic Agglutination Test (MAT) or Indirect Fluorescent Antibody Test (IFAT) diagnostic tests may also be used to detect the Leptospira specific antibodies either.
- The following diagnostic procedures are used to detect *Neospora caninum*: detection of specific antibodies in foetal thoracic or pleural fluid, histopathological examination of the brain or myocardium, immunohistochemistry (IHC) or the use of IFAT or solid phase immunoassay.
- Bovine viral diarrhoea virus is detected using ELISA, PCR or IFAT diagnostic tests.
- A blood sample collected from the dam may also be examined.
- The development stage of the foetus may help confine the diagnostic route to be chosen.

- Knowledge of the vaccination history of the dam is vital to help establish a correct diagnosis and to avoid any diagnosis of false positives.
- In general it is advised to interpret all foetal and maternal serological results with caution during the investigation of bovine abortion.

(Department of Agriculture, Food and the Marine and Agri-Food Biosciences Institute, 2012).

<u>Figure 1:</u> The location of herds (red dots) that submitted bovine carcasses to the AFBI laboratories located at Stormont and Omagh in Northern Ireland during 2011.



Source: All-island Animal Disease Surveillance Report 2011

<u>Figure 2:</u> A map of Ireland showing the location of herds (dots) that submitted carcasses to the Regional Veterinary Laboratories in Ireland during 2011.



Source: All-island Animal Disease Surveillance Report 2011

During the past 18 months a number of private veterinary diagnostic laboratories have established herd health programs to help farmers and veterinarians all over Ireland to determine the levels of disease prevalence in their herds. These laboratories are using bulk milk samples to detect the presence of diseases and each is offering packages to take samples at intervals throughout the year monitoring the progression of pathogens in the herd during the year (McElroy, 2012). In general these laboratories offer screening programs for the following pathogens: *Salmonella spp, Neospora caninum, Leptospira interrogans* serovar hardjo, IBR, BVD, Johnes disease, Fasciola hepatica and Ostertagia (Agri-Food and Biosciences Institute., 2012) These companies are using ELISA (to detect antibodies) and PCR (to detect antigens) as the primary methods of detection. The findings are reported back to the farmer who is then advised to consult with their veterinary practitioner to discuss the results accordingly and organise a vaccination and/or treatment plan for the herd. This method of bulk milk sampling is proving to be a very useful tool to maintain herd health including the identification of pathogens that are causing abortion or may lead to abortion cases if not treated.

3. Results:

During 2011 routine foetal cultures were examined from a combined total of 3,355 foetuses submitted to the laboratories of AFBI and DAFM. The most significant abortifacient isolated was Salmonella Dublin which was detected in a total of 258 cases (Table 1). Numerous other pathogens were isolated from foetal samples. They were considered of lesser importance but nevertheless they have the potential to cause sporadic abortions.

During 2010 similar trends were observed of the primary causative agents of bovine abortion. This year was the first year that veterinary laboratories from the DAFM in Ireland and the laboratories of AFBI in Northern Ireland together produced the All-island Animal Disease Surveillance Report. A combined total of 3,179 fetal cultures were examined during 2010. The figures in Table 1 reflect the most common findings. Seropositivity to L.hardjo, BVDV and *N.Canium* are presented in Table 2.

<u>Table 1:</u> Abortion agents isolated during routine foetal cultures in the AFBI and DAFM in 2011 and 2010.

Agents	2011	2010
	% Positive	% Positive
Salmonella Dublin	7.7	12.9
Arcanobaterium (True-	6.5	5.3
perella) pyogenes		
Bacillus licheniformis	4.1	3.4
Listeria monocytogenes	2.0	1.9
Aspergillus spp.	0.4	1.0
Other microorganisms		
isolated that may cause		
sporadic abortions:		
E. coli and other coli-	8.6	9.8
forms		
Streptococcus spp.	1.9	2.7
Bacillus spp.	0.8	0.6
Staphylococcus spp.	0.5	0.4
Fungal & yeast	0.4	
organisms		
Fungal species		0.3
Pseudomonas spp.	0.4	
Pseudomonas		0.03
aeruginosa		
Pasteurella multocida	0.3	
Pasteurella spp.		0.3
Salmonella spp.	0.3	
Salmonella Typhi-		0.1
murium		
Campylobacter spp.	0.2	0.2
Haemolytic coliforms	0.1	
Listeria spp.	0.1	
Mannheimia	0.1	
haemolytica		
Nocardia spp.	0.1	
Yersinia pseu-	0.1	0.03
dotuberculosis		

Source: All-island Animal Disease Surveillance Reports 2011 & 2010

Infective agents capable of causing abortions were isolated from 20.7% and 24.5% of the foetuses, while 13.9% and 14.5% of the cases bacteria and fungal species were detected, however their pathogenic role can be questioned.

<u>Table 2:</u> Serology from foetal fluids

Agent	2011	2010
DAFM (% positive)		
L. Hardjo	1.60	5.20
BVDV	4.90	6.50
N. caninum	5.30	6.80
AFBI (% positive)		
L. hardjo	4.50	0.90
BVDV	5.40	4.90
N. caninum	7.70	3.20

Source: All-island Animal Disease Surveillance Report 2011

Serological examinations verified the importance of L.hardjo, BVDV and N.caninum.

Salmonella infection continued to be the most significant abortifacient detected during 2010 showing a seasonal increase during the months of October and November (Table 3). Salmonella Dublin and *Brucella abortus* infections can cause abortion storms in herds. While Ireland has brucellosis free status, one fetus was identified with *B.abortus* in Northern Ireland during 2010 (Department of Agriculture, Food and the Marine and Agri-Food Biosciences Institute., 2011). Both *Salmonella spp.* and *B.abortus* can pose a serious zoonotic risk to those in contact with infected animals although S.Dublin rarely carries a risk.

<u>Table 3:</u> Percentage of total foetal cultures per month detected with Salmonella Dublin infection in Ireland (DAFF) and Northern Ireland (AFBI) during 2010

Month	DAFM	AFBI
	%	%
January	5.68	3.17
February	2.18	1.08
March	0.36	2.90
April	4.12	2.17
May	3.23	0.00
June	7.32	2.94
July	10.53	3.85
August	12.00	7.69
September	24.21	19.64
October	36.70	17.78
November	36.16	24.00
December	19.07	0.00
Total % of positive foetal cultures	14.15	7.36

Source: All-island Animal Disease Surveillance Report 2010

During the years 2006 to 2009 the Regional Veterinary Laboratories of Ireland together prepared a Surveillance Report annually. The most common causative agents of bovine abortion and the methods of diagnosis used during these years are illustrated in Tables 4 and 5.

<u>Table 4:</u> Bovine fetal culture results for the years 2006 to 2009 (Department of Agriculture, Food and the Marine., 2010; Department of Agriculture, Food and the Marine., 2009; Department of Agriculture, Food and the Marine., 2008; Department of Agriculture, Food and the Marine., 2007).

Infectious agent	2009	2008	2007	2006
Arcanobacterium	6.7%	5.6%	5.8%	6.9%
(Trueperella) pyogenes				
Salmonella Dublin	6.0%	4.6%	7.0%	6.1%
Bacillus licheniformis	4.0%	2.7%	4.5%	4.1%
Listeria monocytogenes	1.3%	1.6%	1.8%	1.3%
Aspergillus spp.	1.2%	1.2%	0.9%	0.5%
Total no. foetuses examined	2,108	2,014	1,647	1,977

Sources: Regional Veterinary Laboratories Surveillance Reports 2006-2009

<u>Table 5:</u> Infectious causes of abortion detected by serology and other methods (Department of Agriculture, Food and the Marine.,2010; Department of Agriculture, Food and the Marine.,2009; Department of Agriculture, Food and the Marine.,2008; Department of Agriculture, Food and the Marine.,2007).

Agent		2009	2008	2007	2006
Leptospira hardjo	No. tested	521	544	431	428
(serology)	% positive	2.5%	4.0%	3.2%	4.7%
Neospora caninum	No.tested	1282	1080	771	677
•	% positive	4.9%	6.0%	4.9%	5.8%
BVD virus	No.tested	967	812	683	408
(PCR/Antigen ELISA)	% positive	5.5%	5.7%	5.6%	6.4%

Sources: Regional Veterinary Laboratories Surveillance Reports 2006–2009

4. Discussion:

It is seen from the data collected and revealed in the results that there is a broad range of infectious agents responsible for bovine abortions. Abortion in cattle is a common occurrence throughout Europe with particular pathogens associated more commonly with abortions depending on the seasonality and the epidemiological location of the herd. In some countries in Europe there are eradication programs established against some of these abortificants including bovine viral diarrhea virus and infectious bovine rhinotrachitis. During 2013 an eradication program against BVD virus will be implemented in Ireland and so all new born calves must legally be tested for the presence of BVD virus and if a persistently infected (PI) calf is identified this calf must be culled from the herd. Many farms throughout Ireland also implement their own vaccination programs against other abortion causing agents. Most commonly vaccinations against Leptospira, Salmonella, BVD virus and IBR infections are administered in conjunction with biosecurity measures to protect herd health.

4.1 Leptospirosis:

Leptospirosis infection causing bovine abortion has been a common occurrence during the past number of years with high levels of seroprevalance identified in many herds throughout Ireland and Europe. A recent study conducted by Teagasc (2004) and the Department of Agriculture in Ireland found that over 80% of Irish herds are antibody positive for leptospirosis. Leptospirosis in cattle is a zoonotic disease therefore all those in contact with cattle must be aware of the risks of infection including leptospira secretions transmitted in milk which poses as a serious zoonotic risk and so necessary precautions must be undertaken. Those agents responsible for Leptospirosis infection in cattle are L.pomona (originates from pigs), L.grippotyphosa (wild living rodents are the primary hosts) and L.hardjo (infected cattle can be the primary or secondary hosts). Another serovar of the Leptospira genus, L.Bratislava may also cause bovine abortion. A cross sectional study which was completed in Northern Spain identified L. Bratislava as the most prevalent serovar causing abortion in cattle using the microagglutination test for detection (Atxaerandio et al., 2005). The bacteria are most often spread by urine from the infected animals contaminating the environment with rodents regarded as the reservoirs of the bacteria (Djonne, 2007). Leptospira associated abortions in bovines are in most cases(~90%) due to L.hardjo infection. The most common clinical signs of Leptospira infections in cattle are abortions (usually in mid to late pregnancy), birth of weak, dying or dead calves, early embryonic mortality and returns to estrus, poor conception rates as a result of poor infertility, a sudden drop in milk yield which may be observed for fourteen days post infection. But often abortion is the only clinical manifestation which may be seen up to 6 weeks (L.pomona) or up to 12 weeks (L.hardjo) after becoming first infected. Abortion as a result of L.hardjo infection is usually sporadic in nature. Additionally if a cow is persistently infected with L.hardjo, colonization of the uterus and oviducts can occur resulting in infertility characteristics such as decreased conception rate and prolonged calving intervals (Kahn, 2010). The most commonly used diagnostic tests for leptospirosis are the Microscopic Agglutination Test (MAT) or the Fluorescent Antibody Test (FAT). However the interpretation of leptospiral serological results is an issue of contention expressed by diagnostic laboratories because the results can be compromised due to cross-reactivity of antibodies, the presence of antibody titres induced by vaccination and the appropriate cut-off level of antibody titres as a positive indication of infection is not standardized across laboratories in Ireland and the UK. There are vaccines available for use in bovine animals which should be implemented in herds with a known infection and to all those new animals entering a disease free herd. Strict biosecurity measures should be implemented by herd owners to prevent the entry of infection and/or to control the infection including operating a closed herd policy and cutting off areas of communal water supplies and wet areas to decrease the risk of contamination of water sources.

4.2 Bovine viral diarrhea

Bovine viral diarrhea virus is another infectious agent which has been identified as a consistent abortifacient during the past number of years. BVD virus is from the Pestivirus genus with cattle, yak, buffalo and zebu as the main susceptible hosts. If a cow becomes infected with the virus it can reach the foetus through the placenta and depending on the time of infection during the pregnancy the foetus may be aborted or become a persistently infected animal. If the cow is exposed to the virus during the first forty two days of pregnancy early embryonic death occurs and the cow returns to estrus or abortion may occur if the foetus becomes infected after day forty two. The fetus may be expelled as a result or it may be retained and become mummified. However if the foetus is infected during days 42-120 of the pregnancy but does not die the foetus develops immunotolerance and will be born as a persistently infected (PI) calf. In general if the foetus becomes infected during the final trimester the calf will be born with antibodies to BVD (Djonne, 2007). Those calves that are persistently infected with BVD virus and whom are re-infected again during their lifetime develop Mucosal Disease

which has fatal consequences. Diagnosis of BVD virus can be obtained by serological examination of the aborted foetus through ELISA and PCR techniques. In addition bulk milk sampling can be performed in a herd to identify BVD antibodies. BVDV is a major cause of reproductive failure and so eradication programs are in place in many countries across Europe against this disease including the UK and compulsory implementation of a BVD screening program will begin in Ireland in 2013. As many as 80-90% of cattle herds in Ireland have been exposed to BVDV (Sayers, 2012) and so an eradication program should be a very positive progression in order to improve herd health statuses throughout Ireland (Teagasc). All PI animals in a herd will need to be identified and culled but not sold in order for an eradication program to be successful. Often BVDV infection has an immunosuppressive effect on the host and so there is an opening for opportunistic pathogens which are often concurrently isolated with BVDV in aborted fetuses (Department of Agriculture, Food and the Marine and Agri-Food Biosciences Institute., 2012).

4.3 Neospora caninum:

The confirmation of *Neospora caninum* infection as an abortificent is also commonly detected throughout herds in Europe. An expanse of literature has been reported on the infection and its consequences. A recent comparison of the seroprevalence of *N. caninum* in herds across Europe was conducted and it was discovered that the national seroprevalences are between 16 -76% in dairy herds and 41-61% in beef herds (Klevar, 2007). Dogs are the definitive hosts of N.caninum and this infection is passed on to cattle via the dog's faeces containing the excreted oocytes of this parasite which may be accidently ingested by cattle while grazing or by drinking faecal contaminated water. Consequently the infection passes through the placenta (where the tachyzoites develop) and then to the pregnant uterus infecting the foetus where tissue cysts can develop within the central nervous system of the calf. As a result it is important to prevent dogs defecating on pasture to be grazed by the herd or on fodder to be eaten. Also dogs may acquire the infection by ingesting the placenta from an infected bovine thus spreading the infection. The times of infection are termed as an endogenous transplacental infection (TPI) if the foetal infection occurs from a reoccurrence of the maternal infection acquired before pregnancy while exogenous TPI is used to define the infection of the dam during pregnancy (Trees and Williams., 2005). Foetuses exposed to the parasite during early gestation will most likely be aborted while those fetuses exposed later during the pregnancy may survive and appear perfectly normal at birth or have some neurological symptoms that are not

very noticeable (Djonne, 2007). However these calves are now carriers of the infection and should not be used for breeding purposes in the future as the female offspring will most likely abort at some time during their reproductive years continuing the spread of disease. In addition infected cows may abort in consecutive pregnancies. In the immunocompetant foetus the immune system of the foetus slows down the multiplication of tachyzoites which become encysted in the central nervous system and as a result the calf that is born is full term and alive but congenitally infected (Toolan, 2003). Therefore it is important to identify if there is an infection present in the herd and to cull those cattle that are infected and also impose strict biosecurity precautions such as keeping dogs away from birthing pens and do not allow the fetal membranes to be ingested by dogs. Also it should be limited and supervised the amount of access dogs have to the pastures and intended fodder supply of the herd. The methods of detection used for N.caninum include examination of maternal and fetal serology, histopathological examinations and immunohistochemistry methods. In the diagnostic laboratories in Ireland it is the belief that the detection of N.caninum antibodies in a blood sample from a cow which has aborted or detection in the foetal pleural fluid alone is not sufficient to confirm abortion caused by N.caninum infection but a definitive diagnosis is only reached with supporting evidence of protozoal encephalitis or myocarditis during histopathological examination of the foetal tissues (Department of Agriculture, Food and the Marine and Agri-Food Biosciences Institute., 2011)

4.4 Salmonella:

Salmonella infections causing abortion in cattle has emerged as a predominant diagnosis in Ireland for the past number of years. The most common species isolated is S.Dublin but during 2011 other serotypes were isolated such as S.Typhimurium, S.Newport and S.mbandaka (Department of Agriculture, Food and the Marine and Agri-Food Biosciences Institute., 2012). From surveillance data collected in the United Kingdom it was concluded that there is a clear seasonal pattern for Salmonella infections which is most prevalent during late autumn (Animal Health and Veterinary Laboratory Agencies., 2011a). Also it revealed that abortion was a more common clinical finding of S.Dublin infection but this was quite unusual in the case of S.Typhimurium (Carrique-Mas et al., 2010). In Ireland it was observed that a seasonal peak of S.Dublin causing abortion has been highest in November during the past number of years. S.Dublin is identified as a contagious abortion causing agent and abortion storms in Irish herds are often reported as a consequence of this infection in a herd (Department of Agriculture, Food and the Marine and Agri-Food Biosciences Institute., 2012). It has been men-

tioned that drying off cows may act as a predisposing factor leading to the precipitation of a latent S.Dublin infection present in the herd (Crilly., 2004). Salmonella spp. are zoonotic, however the mostly commonly isolated spp. in this case S.Dublin is not associated as high a zoonotic risk unlike S.Typhimurium but it must be noted that if humans do become infected with S.Dublin, the case fatality rate of the septicaemic form of the resulting disease in the elderly is 15% (Crilly., 2004). Sometimes cows infected with a Salmonella spp. induced abortion may also show signs of enteritis and septicemia but more often than not abortion is the only clinical symptom. Salmonella induced abortions in bovine animal's peak during the seventh and eighth months of pregnancy. The aborted foetus is often mummified with a distinct odor. On closer post mortem examination, the foetus is often discovered to have undergone autolysis and become emphysematous. Diagnosis is most often achieved by serological examination of the abomasal contents of the foetus. As a result of the seasonal pattern of Salmonella related abortions it is an important factor to vaccinate all animals in the herd during the correct time of year to provide optimal protection against this pathogen in which case the vaccinations should be administered in August or September. Another important preventative measure against the spread of the disease is to avoid the disposal of faeces collected from the winter housing of those animals known to have Salmonella infection as the pathogen is spread by the faecal oral route and once spread on the land it may survive for 12 months (Crilly, 2004).

4.5 Arcanobacter (Trueperella) pyogenes:

Arcanobacter pyogenes (recently classified as Trueperella pyogenes) has been diagnosed as a causative agent of sporadic abortions in Irish herds. During a study compiled by Teagasc (2004) in Ireland A. (T.) pyogenes was identified as an isolate quite regularly from cases of abortion/stillbirth examined from the years 1989-2003. On average the highest incidence of A. (T.) pyogenes related abortion was recorded during the month of January accounting for 6.1% of pathogens isolated (Teagasc, 2004). A. (T.) pyogenes is an omnipresent organism in the environment often present in abscesses or in cases of mastitis or pneumonia which may develop into a bacteraemia thus leading to endometritis, placentitis and subsequent abortion. While the infection may not cause any symptoms in the cow the foetus is more susceptible mostly because of its immature immune system (Djonne., 2007). As a result of placentitis this is a predisposing factor to foetal hypoxia and on examination the foetus is usually autolyzed, with fibrinous pericarditis, pleuritis or peritonitis also possible. Unfortunately no effective bacterin is available as a preventative measure (Kahn, 2010).

4.6 Brucellosis:

Brucellosis caused by Brucella abortus, Brucella mellitensis or Brucella suis are notifiable diseases according to the OIE listed diseases (OIE, 2012). It is a zoonotic disease and so eradication programs against the disease have been established throughout Europe. All foetal samples and maternal blood samples submitted for examination as a result of abortion are routinely examined for brucella infection in diagnostic veterinary laboratories across Ireland. B. abortus has not been identified in the Republic of Ireland since 2005. However one case of B. abortus was identified in a foetus in Northern Ireland during 2010 and so highlights the necessity to maintain vigilant screening programs and diagnosis of the disease (Department of Agriculture, Food and the Marine and Agri-Food Biosciences Institute., 2011). Brucella infection is acquired by the per oral route, reaching the lymph nodes, causing a septicemic infection and the bacteria settles in the predilection organs of the pregnant uterus, placenta and udder. Consequently placentitis develops and necrosis in the chorionic villi occurs resulting in abortion and retained foetal membranes due to adhesions present intrauterine with abortion taking place during the sixth to eighth months of gestation. Clinical symptoms of the impending abortion are vulvovaginitis and greyish-white/red colored vaginal discharge present one to two days before abortion. Transmission of the infection occurs via infected milk or infection carried by means of transport or footwear. Transmission among the animals of a herd results from contact with infected placenta, foetal membranes and vaginal discharge. During post mortem examination of the aborted foetus, oedema and haemorrhages are observed in the carcass as well as small necrotic foci in the liver. The fetal membranes have obvious pathological lesions of placentitis, hyperaemia, oedema and necrotic lesions. Diagnosis of this disease is achieved by examination of the whole foetus and of a maternal blood sample. Many methods of diagnosis can be utilized including identification of the pathogen using a direct smear and Koster staining or serological examination with the use of Complement Fixation Test (CF) or ELISA methods. PCR may also be used as a method of detection. During a recent study conducted in Turkey it was found that the PCR detection kit used resulted in 83% sensitivity and 94% specificity for the pathogen (Buyukcangaz et al., 2011). Due to the zoonotic nature of this disease and its contagious abilities all those animals diagnosed with the disease must be isolated from the remainder of the herd and culled as soon as possible. During the late 1990's the Department of Agriculture in Ireland imposed a series of measures to enhance the eradication of brucellosis from the country including pre-movement testing, an intensified annual testing program of all eligible bovine animals and rapid depopulation policy to avoid contiguous spread of the disease. Additionally, measures to treat faecal waste of all those herds infected before it is disposed on the land as a fertilizer was also introduced and imposed by the Irish Government (Sheahan et al., 2006). The implemented measures radically reduced the infection rate of *B. abortus* in Irish herds and propelled Ireland towards impending brucellosis free status.

4.7 Infectious bovine rhinotracheitis:

One of the clinical manifestations of infectious bovine rhinotracheitis (IBR) infection is also abortion. This abortifacient is caused by bovine herpesvirus-1. This agent is present world-wide however some countries in Europe have eradicated the disease successfully. The occurrence of IBR in Ireland was investigated and the seroprevalance of BHV-1 in herds was found to be 74.9% with no significant differences between dairy and beef herds (Cowley et al., 2011). BHV-1 was found to be significantly related to reproductive performance in adult dairy cattle when examined (Raaperi., 2012). Abortion occurs as a result of viraemia which penetrates to the placenta and the uterus. The foetus is usually infected between 4 months and the end of term. Autolysis of the foetus is a consistent feature of this infection. Vaccination programs against this disease are followed in many herds with the use of live marker vaccines proving very effective.

4.8 Chlamydophilosis:

Chlamydophila abortus causing chlamydophilosis is another sporadic abortifacient diagnosed in cattle. During Spring 2011 in Scotland 18.6% of placenta samples submitted for examination had evidence of Chlamydia-related organisms (Wheelhouse et al., 2012). Methods of detection of these agents include PCR, molecular and immunohistochemistry methods and histopathological examinations with purulent to necrotizing placentitis being a prominent histopathological feature during examination of Parachlamydia abortion (Ruhl et al., 2009). Coxiella burnetii has also been detected from placenta and fetal samples with the use of PCR as a method of detection (Jones et al., 2010). Streptococcus pluranimalium is another pathogen that has been recognized as a possible cause of bovine abortion (Foster et al., 2008). Additionally E.coli and Campylobacter bacterial agents have been isolated in foetal cultures submitted to veterinary diagnostic laboratories in Ireland during recent years (Department of Agriculture, Food and the Marine and Agri-Food Biosciences Institute., 2012).

4.9 Other bacterial agents:

Other bacterial agents that were isolated from foetal cultures examined in veterinary laboratories throughout Ireland include Bacillus licheniformes and Listeria monocytogenes. Both these bacterial agents can be acquired from the ingestion of contaminated feed with sporadic abortions in a herd the resulting consequence. B.licheniformes thrives in spoiled forage and feed, however its pathogenic nature can be questioned. It has been diagnosed on average in 3.8% of all foetal cultures submitted to veterinary laboratories in Ireland during the years 2006-2011. The pathological lesion observed at post mortem examination of the foetus is typically placentitis with thickening of the allantochorion in a non-autolyzed foetus. Unfortunately due to the ubiquitous nature of this pathogen it is not feasible to control or implicate preventative measures. Abortions resulting from the ingestion of infected fodder are also a consequence of L.monocytogenes infection. Silage that is poor quality or spoilt with large amounts of the pathogen L. monocytogens is most commonly the source of infection. Discharge from an aborting cow can also pertain as source of infection. A bacteriaemia develops in the infected dam and as a result gains access to the uterus and foetus. The resulting abortion is usually sporadic in occurrence and occurs during late gestation (Djonne., 2007). The highest number of incidences relating to this pathogen is reported during the winter months when silage is typically fed to cattle. During the years 2006 to 2011, the pathogen was diagnosed on average in 1.7% of all foetal cultures examined in Ireland during this time (Department of Agriculture, Food and the Marine and Agri-Food Biosciences Institute., 2012). The resulting aborted foetus may have slight to marked autolysis, small necrotic foci are visible on the liver. Upon examination of the gram stained abomasal contents the typical pleomorphic coccobacilli are observed (Kahn, 2010). If a suspicion of bacterial contamination of silage is revealed, a sample of the fodder should be sent for examination and this feed source removed from the herd if necessary.

4.10 Mycotic infections:

Mycotic infections causing abortion in cattle are also a reported finding each year in Irish herds. However their reported incidence is quite low with an average of 0.8% between the years 2006 to 2011 (Department of Agriculture, Food and the Marine and Agri-Food Biosciences Institute., 2012). Most often mycotic related abortions occur during the last two months of gestation (Djonne, 2007) with *Aspergillus spp.* as the most commonly identified source of these infections. Abortions caused by this fungal species have characteristic lesions when ex-

amined post mortem. This infection is also spread via a hematogenous route typically infecting the cotyledonary areas of the placenta at first then proceeding to the intercotyledonary areas resulting in necrosis of the affected areas. Similar to *Listeria monocytogenes* infection, *Aspergillus spp.* infections are more commonly identified during the winter while the cattle are housed indoors and possibly exposed to moldy fodder. Skin lesions are reported in approximately 30% of aborted fetuses infected with a mycotic agent. Diagnosis is achieved by the presence of fungal hyphae collected from the stomach contents, skin lesions or placenta. It is advised to avoid feeding moldy hay or silage as a preventative measure.

4.11 Other infectious agents:

Many other infectious agents have been isolated from aborted fetuses in Ireland and Europe. Bacterial agents appear to be the most commonly identified pathogens when diagnosing abortions. An uncommon but identifiable abortifacient is from the genus's Mycoplasma and Ureaplasma. These microorganisms are ubiquitous in the environment and are often located in the mucosa of the orifices of the body including the oral and nasal cavities, vulva and penile urethra. In the United Kingdom during 2011 Mycoplasma bovirhinis and Ureaplasma diversum were diagnosed as causes of sporadic abortion (Animal Health and Veterinary Laboratory Agencies., 2011b). *Ureaplasma diverticulum* is associated with granular vulvovaginitis which can predispose to infertility, sporadic abortions and neonatal mortality (Radostits, 2007). In Finland, Ureaplasma diverticulum has been diagnosed as one of the most common causes of sporadic abortions in cattle (Syrjala, 2007). In the United Kingdom, the Animal Health and Veterinary Laboratories Agency (AHVLA) standard laboratory protocols for bovine abortion do not usually include examinations necessary to identify these microorganisms. However due to the possibility of these pathogens as a new emerging disease it has been brought to attention that the AHVLA should include the examination of these pathogens in their surveillance role of bovine abortions in the future (Murray, 2012).

Many microorganisms can be cultured from aborted fetuses but these organisms may also be cultured from the tissues of healthy animals suggesting that their presence in an aborted fetus alone cannot be considered as a definitive diagnosis for abortion but supporting evidence must also be collected such as the frequency of occurrence and herd history to present a diagnosis.

5. Summary:

Importance and frequency of bovine abortions in Ireland was analysed using the data of Department of Agriculture, Food and the Marine, the Regional Veterinary Laboratories located throughout Ireland and the Agri-Food and Biosciences Institute in Northern Ireland. Infective agents capable of causing abortions were isolated from 20.7% and 24.5% of the foetuses, while in further 13.9% and 14.5% of the cases bacteria and fungal species were detected, however their pathogenic role can be questioned. Serological examinations verified the importance L. hardjo, BVDV and *N. caninum*. The data confirmed the dominance of Salmonellae in the aetiology of bovine abortions in Ireland. The analysis of the results underlines the importance of maintaining the surveillance work in the field of abortions.

Összefoglalás

A Mezőgazdasági, Élelmiszer- és Tengerészeti Minisztérium, az írországi területi állategészségügyi laboratóriumok, valamint az észak-írországi Agrár-Élelmiszer- és Biotudományi Intézet adatainak felhasználásával az írországi marhavetélések gyakoriságát és oktanát elemezték. A magzatok 20,7% illetve 24,5%-ából izoláltak olyan kórokozót, amely vetélést tud okozni, míg az esetek további 13,9% illetve 14.5%-ából is baktériumokat és gombákat mutattak ki, bár ezek kórtani szerepe kérdéses. Szerológia vizsgálatok igazolták a L. hardjo, a BVDV és a *N. caninum* jelentőségét. Az adatok megerősítették, hogy az írországi vetélések oktanában a salmonellák leggyakoribb kórokozók. Az adatok elemzése aláhúzza, hogy nagyon fontos fenntartani a vetélések oktani vizsgálatát.

6. References:

Agri-Food and Biosciences Institute. 2012. Milk testing. URL: http://www.afbini.gov.uk/bulk-tank-milk-testing.pdf Downloaded on: October 2012.

Animal Health and Veterinary Laboratory Agencies. 2011. Emerging threats cattle quarterly reports October – December 2011. United Kingdom. URL: http://vla.defra.gov.uk/reports/docs/rep_survrep_qtlyc0411.pdf Downloaded on: October 2012.

Animal Health and Veterinary Laboratory Agencies. 2011. Salmonella in Livestock Production in GB. Chapter 2. Reports of Salmonella in Cattle. URL: http://www.veterinaryirelandjournal.com/images/stories/pdfs/la/la_jun_2012.pdf Downloaded on: October 2012.

Atxaerandio, R., Aduriz, G., Ziluaga, I., Esteban, J.I., Maranda, L., Mainar-Jaime, R.C. 2005. Serological evidence of Leptospira interrogans serovar Bratislava infection and its association with abortions in cattle in northern Spain. *Veterinary Record*; 156:376-380

Buyukcangaz, E., Sen, A., Carli, K.T., Kahya, S. 2011. Comparison of direct culture versus PCR for the detection of Brucella in aborted foetuses of cattle and sheep in Turkey. *Veterinary Record*;168:430

Cabell, E. 2007. Bovine abortion: aetiology and investigations. *In Practice*; 29:455-463

Carrique-Mas, J.J., Willmington, J.A., Papadopoulou, C., Watson, E.N., Davies, R.H. 2010. Salmonella infection in cattle in Great Britain, 2003 to 2008. *Veterinary Record*; 167:560-565

Cattle Health Certifications Standard. July 2012. United Kingdom. Incorporating rules for cattle herd schemes. URL:

http://www.checs.co.uk/media/pdf/CHeCS_Tech_doc_final_Version_2012-1.pdf Downloaded on: October 2012.

Cowley,B.DJ., Clegg,T., Doherty,L., More,S.J. 2011. Aspects of bovine herpesvirus-1 infection in dairy and beef herds in the Republic of Ireland. *Acta Veterinaria Scandinavica*;53:40.

Crilly,J. The Epidemiology of Bovine Salmonellosis in Cork and Kerry. Teagasc 2004. URL: http://www.teagasc.ie/research/reports/dairyproduction/4992/eopr-4992.asp Downloaded on: 10th October 2012.

Department of Agriculture, Food and the Marine, Agri-Food Biosciences Institute. 2012. Allisland Animal Disease Surveillance Report 2011. PDF: 2011 AFBI/DAFM All-Island Surveillance Report Downloaded on: October 2012

Department of Agriculture, Food and the Marine, Agri-Food Biosciences Institute. 2011. Allisland Animal Disease Surveillance Report 2010. PDF: 2010 AFBI/DAFF All-island Surveillance Report Downloaded on: May 2012

Department of Agriculture, Food and the Marine. 2007. Regional Veterinary Laboratories Surveillance Report 2006. PDF: <u>Veterinary Laboratories Disease Surveillance Report 2006</u>
Downloaded on: May 2012

Department of Agriculture, Food and the Marine. 2008. Regional Veterinary Laboratories Surveillance Report 2007. PDF: <u>Regional Veterinary Laboratories Surveillance Report 2007</u>
Downloaded on: May 2012

Department of Agriculture, Food and the Marine. 2009. Regional Veterinary Laboratories Surveillance Report 2008. PDF: <u>Regional Veterinary Laboratories Surveillance Report 2008</u>
Downloaded on: May 2012

Department of Agriculture, Food and the Marine. 2010. Regional Veterinary Laboratories Surveillance Report 2009. PDF: <u>Regional Veterinary Laboratories Surveillance Report 2009</u>
Downloaded on: May 2012

Department of Agriculture, Food and the Marine. Laboratory Services. URL: http://www.agriculture.gov.ie/animalhealthwelfare/laboratoryservices/regionalveterinarylaboratories/packaging/ Downloaded on: 8th October 2012.

Djonne,B. 2007. Infectious and perinatal diseases – a comparative overview. *Acta Veterinaria Scandinavica*;49(Suppl 1):S10

Foster, G., Barley, J., Howie, F., Falsen, E., Moore, E., Twomey, D.F., Wragg, P., Whatmore, A.M., Stubberfield, E. 2008. Streptococcus pluranimalium in bovine reproductive disease. *Veterinary Record*; 163:638.

Jones, R.M., Twomey, D.F., Hannon, S., Errington, J., Pritchard, G.C., Sawyer, J. 2010. Detection of Coxiella burnetii in placenta and abortion samples from British ruminants using real time PCR. *Veterinary Record*; 167:965-967.

Kahn, M.C. (ed.) 2010. Merck Veterinary Manual 10th edition. p.1225-1229

Klevar, Siv. 2007. Tissue cyst forming coccidian; Toxoplasma gondii and Neospora caninum as a course of disease in farm animals. *Acta Veterinaria Scandinavica*;49(Suppl 1):S1.

Maxie, M,G.(ed). 2007. Jubb, Kennedy and Palmer's Pathology of Domestic Animals Volume 3. 5thedition. p.474-480

McElroy, S. 2012. Bulk Milk disease Screening – A useful tool in assessing infectious disease status in Irish dairy herds. *Irish Veterinary Journal*. URL: http://www.veterinaryirelandjournal.com/images/stories/pdfs/la/la_jun_2012.pdf Downloaded on: October 2012.

McGavin, M.D., Carlton, W.W., Zachary J.F. 2001. Thomson's Special Veterinary Pathology. 3rdedition. p.615

McGavin, M.D., Zachary, J.F. 2007. Pathological Basis of Veterinary Disease. 4th edition. p.1294

Murray, Richard.D. Laboratory Diagnosis of Mycoplasma/Ureaplasma abortion in cattle. 2012. *Veterinary Record*;170:130-131.

OIE World Organisation for Animal Health. 2012. OIE listed diseases 2012. URL: http://www.oie.int/animal-health-in-the-world/oie-listed-diseases-2012/ Downloaded on: October 2012.

Raaperi, K., Bougeard, S., Aleksejev, A., Orro, T., Viltrop, A. 2012. Association of herd BRSV and BHV-1 seroprevalence with respiratory disease and reproductive performance in adult dairy cattle. *Acta Veterinaria Scandinavica*; 54:4.

Radostits, O. 2007. Veterinary Medicine 10th edition, p.1125.

Ruhl, S., Casson, N., Kaiser, C. 2009. Evidence for Parachlamydia in bovine abortion. *Veterinary Microbiology*;135:169-174.

Sayers,R. 2012. BVD- What it is and how to control it. Teagasc. URL: http://www.agresearch.teagasc.ie/moorepark/Articles/BVD.pdf Downloaded on: October 2012

Sheahan, M., O'Hagan, G., Power, S., Kenny, K. 2006. Brucellosis in cattle in Ireland 1998-2005: Progress towards eradication continues. *Irish Veterinary Journal*; 59:217-221.

Syrjala, P., Marjukka, A., Dillard, K., Fossi, M., Collin, K., Nylund, M., Autio, T. 2007. Causes of bovine abortion, stillbirth and neonatal death in Finland 1999-2006. *Acta Veterinaria Scandinavica*, 49(Suppl):S3.

Teagasc. 2004. Seasonality of S. Dublin, A. pyogenes and L. monocytogenes isolates in abortion/stillbirth submissions, Cork RVL, 1989 – 2003. URL: http://www.teagasc.ie/research/reports/dairyproduction/4992/hyper18abortseatab.asp
Downloaded on: 10th October 2012.

Toolan,D. 2003.Neospora caninum abortion in cattle – a clinical perspective. *Irish Veterinary Journal* August;56(8):404-410

Trees, AJ., Williams, DJ. 2005. Endogenous and exogenous infection in Neospora caninum and Toxoplasma gondii. *Trends Parasitol* 2005 Dec;21(12):558-61. Epub 2005 Oct 11.

Wheelhouse, N., Howie, F., Gidlow, J., Greub, G., Dagleish, M., Longbottom, D. 2012. Involvement of Parachlamydia in bovine abortions in Scotland. *The Veterinary Journal*; 193(2)586-588.

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