Chapter 3

DIFFERENTIAL DIAGNOSIS OF INFECTIOUS ABORTION IN SMALL RUMINANTS

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3.1 SUMMARY

Infectious abortion in small ruminants can be bacterial, viral or parasitological in origin. The relative importance of the different aetiological agents depends on the local epidemiological conditions. Multiple infections often occur.

Direct diagnosis involves the isolation and identification of the pathogenic agents present in samples from the aborted foetus, the placenta or vaginal secretions. A selection of techniques can be applied to samples to detect the endemic infectious agents. These techniques include direct examination and cultural methods adapted to the nature of the infectious agents being detected.

Indirect diagnosis, carried out on serum samples taken from the aborted female, uses serological techniques to detect specific antibodies to the abortive agents that occur most frequently in the considered region. It is necessary to analyse a number of samples to confirm the nature of the infection and establish the enzootic character.

Determination of the cause of infectious abortion can only be achieved by differential diagnosis. The number and type of analyses undertaken depends on the nature of the infectious agents studied with regard to the local epidemiological situation and the nature of the samples recovered. The analytical capability of the laboratory and financial and sanitary criteria mean that studies are sometimes limited, in the first instance, to the diagnosis of the main abortive diseases of bacterial origin. Other infections are only studied in the second instance. Current development of new techniques will probably allow the production of standard analytical techniques better adapted to differing epidemiological situations in the near future.

3.2 INTRODUCTION

Infectious abortion usually occurs during the last three months of gestation. Abortions are generally of an enzootic nature and several potentially abortive diseases are able to co-exist. These diseases are caused by bacteria, viruses or parasites which colonise the genital region particularly during gestation. They cause premature expulsion of the foetus and sometimes, depending on the aetiology, illness and even death of the dam. The gross pathology at necropsy is rarely indicative of a precise aetiology, diagnosis is essentially differential, and tests must be carried out to distinguish latent infections from the advanced infection responsible for the observed abortions.

Direct diagnosis, undertaken on samples of the products of the abortion, is sometimes sufficiently conclusive to allow the immediate establishment of sanitary measures or treatments designed to limit the clinical and economic consequences of the concurrent infection. Indirect diagnosis, often undertaken

after the abortion, allows implementation of prophylactic measures, sanitary or vaccinal, relevant to the epidemiological conditions and the local legislation concerning the different infections.

3.3 SAMPLES

In addition to the identification of the cause of infectious abortions, diagnosis should establish the enzootic nature of the disease. Analyses for the main abortive diseases should therefore be undertaken on several samples by direct and indirect methods. Samples should include several aborted foetuses and their associated membranes (Figure 3.1).

Most of the pathogenic agents can be effectively isolated from the organs of aborted foetuses but some agents can be detected more easily in the placenta. Vaginal samples (swabs, tampons, scrapings) can also be used but they must be collected as soon as possible after abortion [10].

3.4 RISKS TO HUMAN HEALTH

To prevent the risk of human infection, samples taken from abortions must be handled with all the precautions necessary to protect personnel and to avoid contamination of the environment.

3.5 DIRECT DIAGNOSIS

Direct diagnosis involves the application of a group of methods designed to simultaneously detect the potentially abortive infectious agents that occur most frequently in a given region (Figure 3.2). These techniques include direct examinations and culture methods adapted to the nature of the samples and the infectious agents. In non-specialised analytical laboratories investigations are often limited to detection of the most common bacterial causes of abortion.

Virological, histological and parasitological investigations are generally only undertaken in more specialised laboratories when such agents are strongly suspected of being involved.

3.5.1 Bacteriological examination

Under certain conditions simple bacteriological testing is all that is required to detect some of the major causes of abortion (rickettsiosis, campylobacterosis, etc.). Confirmation of a speculative diagnosis by isolation of the organism in culture can lead to the rapid introduction of medicinal or sanitary measures to control the infection. Identification of the species of bacteria isolated is achieved by application of a number of differential staining methods although, in recent years, more specific immunohistological and immunoenzymatic techniques have been introduced.

3.5.1.1 Placenta

Smears from cotyledons should be mounted on grease-free slides. Since the cotyledons are unlikely to be uniformly infected it is best to undertake a single analysis on as many cotyledons as possible (5 to 10) rather than take multiple samples from a single cotyledon.

A smear of the vaginal sample should be made in the absence of the placenta.

Note

• If neither the placenta nor a vaginal sample have been collected it is sometimes possible to obtain a smear from the mucus of the aborted foetus or to take a scraping from the umbilical cord. Testing of these samples is, however, generally less effective.

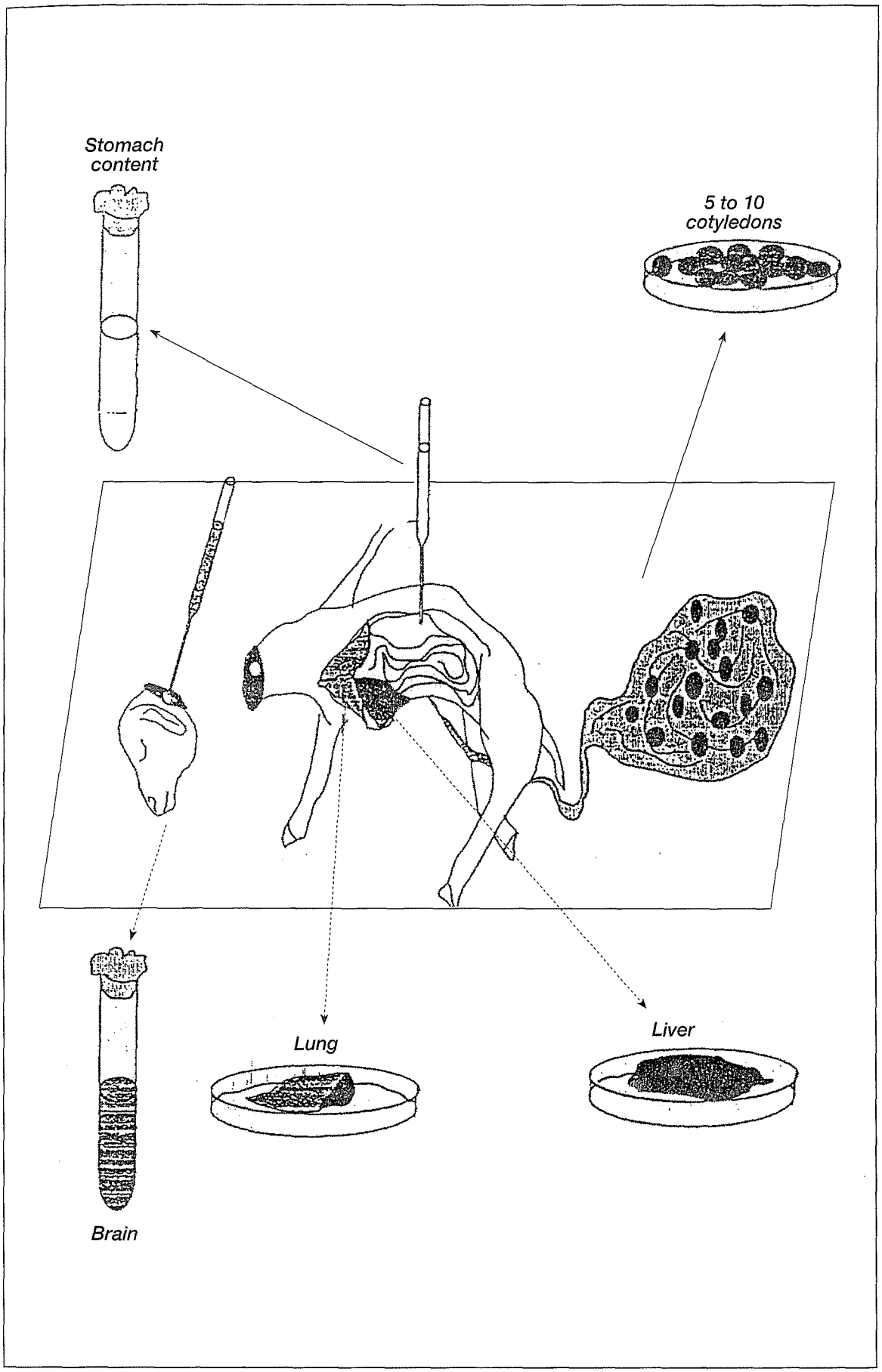

Figure 3.1 : Bacteriological diagonsis of infectious abortions. Sample collection.

3.5.1.2 Stomach contents

Fresh samples of the stomach contents of the aborted foetus can be examined directly on a slide against a black background or by phase contrast to show the presence and characteristic motility of certain infectious agents (for example, *Campylobacter*). Two or three smears should also be made on grease-free slides, one of which should be reserved for immunoenzymatic analysis.

3.5.1.3 Staining

Sections of cotyledons and smears should be submitted for differential staining. For example, the Stamp stain is used to distinguish *Brucella, Chlamydia* and *Coxiella* (Figure 3.3, page 89) ; the Vago stain, or more simply the Gram stain, counterstained with fuchsin allows detection of *Campylobacter* in smears taken from the stomach contents. Other stains can be applied (Koster, Machiavello, May Grunwald Giemsa, etc) provided that the results can be interpreted with confidence.

Immunoenzymatic or gene amplification (PCR) techniques can be applied to all types of samples. They will probably eventually provide an alternative to the previously described techniques which could lead to direct and more specific control of a number of infectious agents.

3.5.2 Culture methods

3.5.2.1 Foetal organs

Foetal organs (e.g. brain, liver) and the stomach contents should be systematically seeded into non-specific nutrient media (e.g. nutrient agar or broth, Tryptose soya). These media allow the isolation of common bacteria (enterobacteria or others) which cause abortion under certain conditions, as well as more specifically abortive bacteria that have simple culturing requirements and are sufficiently abundant in the products of abortion (*Salmonella, Listeria*). The seeding into specific or selective media allows the culture of

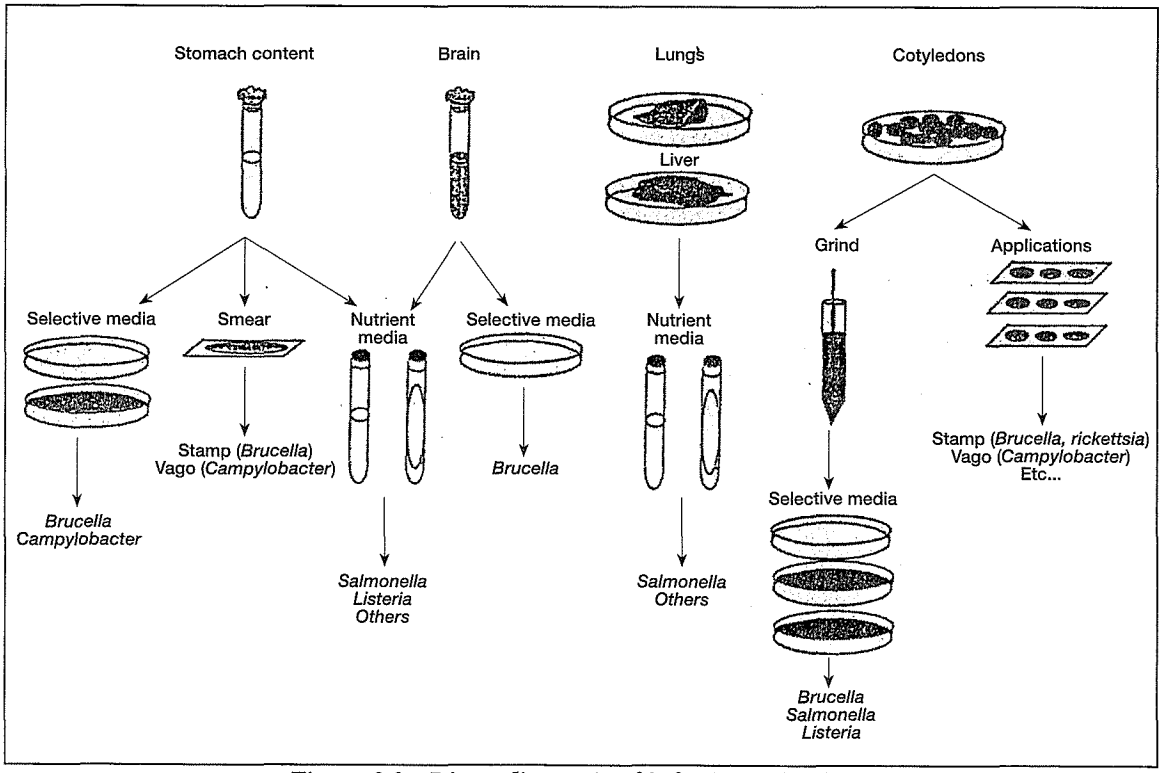

Figure 3.2 : Direct diagnosis of infectious abortions. Analysis of samples

more fastidious abortive bacteria (*Brucella, Campylobacter, Leptospira, Yersinia*).

3.5.2.2 Placenta

Ground samples of cotyledons or vaginal samples, usually more heavily infected than the foetal organs, should be seeded into selective media for the detection of the more common abortive bacteria (*Brucella, Salmonella, Listeria,* etc.). These samples are usually sufficiently infected to produce abundant cultures and do not require the use of enrichment media. Enrichment media may also allow the isolation of bacteria of environmental origin (for example *Listeria*) and so lead to erroneous results.

It is possible to detect two potentially abortive agents in the same sample in areas of multiple infections (for example: isolation of *Salmonella* or of *Brucella* and detection of *Chlamydia*). The overall flock diagnosis is then employed to identify the agent responsible for the observed abortions, the presence of the other perhaps indicating a latent infection.

Testing in sensitive cell cultures (Border disease, rickettsia), histological and immunohistological examination (*Toxoplasma*, mycosis), immunoenzymatic [1, 4, 7] or other [12] techniques are not usually undertaken systematically in routine diagnosis. Such methods are only undertaken when the involvement of a specific agent is strongly suspected or when previous tests have not identified the cause of the abortions. Some of these infections are most easily detected by serodiagnosis, often more rapidly and at lower cost.

3.5.3 Interpretation of results and limitations

The proposed steps allow detection of the main bacterial abortive diseases by use of simple techniques. The effectiveness of the testing, however, depends on the quality of the samples. The relevance of such testing is highest when certain bacterial diseases predominate (brucellosis, salmonellosis, listeriosis, etc.) and decreases considerably when samples are restricted (for example in the absence of the placenta) or when viral (pestiviruses) or parasitological (toxoplasmosis) infections predominate [8, 13]. Taking these factors into consideration, a positive result is always proof of the cause of infection but a negative result does not prove an agent was not involved. Detection of an abortive agent in a single sample does not necessarily confirm the cause of the observed enzootic disease; all individual results must be backed up by flock diagnosis or a serological test which provides confirmation of the enzootic nature of the infection and after other causes of abortion have been ruled out in preceding tests.

It is therefore imperative that the analytical report includes a statement of the effectiveness of the different tests and gives the details of their respective results (Table 3.1).

Table 3.1 : Types of analytical reports of direct diagnosis of infections abortions

Direct examination : Stamp stain : **positive** (presence of rickettsia bodies) **Cultures** : Test for *Salmonella* : negative Test for *Listeria* : negative Test for *Campylobacter* : negative Test for *Brucella* : negative **Interpretation : Chlamydiosis suspected**
Direct examination : Stamp stain : **negative** **Cultures** : Test for *Salmonella* : negative Test for *Campylobacter* : negative Test for *Brucella* : negative Test for *Listeria* : positive ***Listeria monocytogenes*** : isolated from the brain of the aborted fœtus **Interpretation : *Listeriosis***

3.6 INDIRECT DIAGNOSIS

Serological diagnosis is used either to confirm the findings of direct diagnosis or is carried out as an independent analysis after abortions have taken place. It consists of techniques intended to simultaneously detect specific antibodies to the main abortive agents.

3.6.1 Principle

The serological diagnosis of infectious abortions is inevitably a comparative diagnosis applied to the flock. In the Mediterranean region, for example, simultaneous testing should be undertaken for brucellosis, salmonellosis, chlamydiosis and Q fever. Other analyses are only then undertaken if these initial tests prove negative or for identification of the source of infection.

The choice of techniques used depends on the nature of the infectious agent, on the techniques that can be undertaken in the laboratory and on the local legislation regarding the handling of contagious organisms. Traditional techniques such as seroagglutination, complement fixation or immunofluorescence recommended by the O.I.E. [9] generally give satisfactory results in the serodiagnosis of abortions. The only requirement demanded of the test used is that it gives quantitative results. In areas of multiple infection, serodiagnosis is the only effective way to differentiate between a vaccination or latent infection and the infection responsible for the observed abortions. Abortions or low productivity frequently produce an anamnestic response which is expressed by an overall boost in the rate of serum antibody production [14]. The interpretation depends on a comparative analysis of the results obtained with different antigens.

3.6.2 Interpretation of results and limitations

To interpret serological results, details of the effectiveness of the tests carried out and the individual results as titres or as dilution factors of the sera tested must be included in the report (Table 3.2). Depending on the criteria adopted, the results of the analyses can be interpreted at two levels.

- Firstly, for serodiagnosis of infectious abortion and if samples are taken less than 8 weeks after the abortions :

Table 3.2 : Example of the report of serodiagnosis of infectious abortions

Sera	Brucellosis (1)	Chlamydiosis (1)	Q fever (1)	Salmonellosis (2)	Toxoplasmosis (3)
1	0	1/10	0	**1/160**	0
2	0	1/20	0	**1/1280**	0
3	0	1/10	1/5	**1/5120**	0
4	0	0	0	**0**	0
5	0	0	1/10	**1/10240**	0
6	0	1/20	1/10	**1/640**	0
7	0	1/10	0	**1/1280**	0
8	0	0	0	**1/2560**	0
9	0	0	1/20	**1/2560**	0
10	0	1/40	0	**1/640**	0

1: Complement fixation test *2 : Seroagglutination test* *3 : ELISA*

The most probable cause of the observed abortion is the organism for which the greatest number of sera present antibody levels higher than the threshold level for the method used.

- Secondly, during sero-epidemiological investigations, interpretation can be made in terms of flock infection. The best time for samples to be taken is during the period 4 to 8 weeks after low productivity.

The flock is considered to have a latent infection (or recent vaccination) for the disease when steady antibody levels higher than the treshold for the methods used are recorder.
It is considered that no contact with the pathogens has occurred when all sera tested give results below the threshold.

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In the example in Table 3.2, the abortions may be attributed to a *Salmonella* infection; the flock is latently infected with *Chlamydia* and Q fever; the flock is presumed to have had no contact with *Brucella* and *Toxoplasma* (the uninfected status can only be confirmed by two successful tests undertaken on all animals in the flock).

3.7 CONCLUSIONS AND FUTURE WORK

The cause of infectious abortion can only be ascertained by careful differential diagnosis. From a good knowledge of the main local pathogens, which allows screening to be limited, a group of analyses can be undertaken which, in most cases, can be carried out in non-specialised analytical laboratories.

Direct diagnosis allows detection of most of the major bacteriological causes of abortion. Indirect diagnosis, used alone or as confirmation of direct diagnosis, allows establishment of the enzootic nature of infections and determination of

Table 3.3 : Comparative summary of diagnostic methods for determination of infectious abortions

Infectious abortions	Direct diagnosis		Indirect diagnosis
	Direct examination	Culture	Serological testing
Brucellosis	+	+	+
Salmonellosis	-	+	+
Listeriosis	-	+	(+) [a]
Campylobacterosis	+	+	(+) [a]
Chlamydiosis	+	(+) [c]	+
Q fever	+	(+) [c]	+
Leptospirosis	+		(+) [a]
Other bacteria	-	+	(-) [b]
Toxoplasmosis	(+) [b]	-	+
Viral diseases	-	(+) [c]	(+) [d]

(a) : following confirmation as the source of infection
(b) : immunoenzymatic or immunohistological techniques
(c) : sensitive cell culture or immunoenzymatic techniques
(d) : seroneutralisation in sensitive cell cultures

certain non-bacteriological infections (Table 3.3). Serological tests can also be applied to heart blood, pleural or peritoneal fluids of the aborted foetus. However, the foetus will not have developed the ability to produce antibodies if the infection is very severe or occurs very early in gestation and a negative result in such cases would not exclude that organism as the cause of infection.

Development of immunoenzymatic techniques may allow future use of a technique similar to that of the ELISA for all indirect diagnosis, providing that there is a sufficiently consistent system of interpretation of results. Advancement of knowledge in this area in the last few years and, in particular, the emergence and expansion of immunohistological techniques and those of gene amplification may allow the production of future diagnostic techniques which are more rapid, more specific and better adapted to different local epidemiological situations.

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