A FIELD STUDY OF PLACENTAL AND FETAL LESIONS ASSOCIATED WITH BOVINE ABORTION.

R.D.MURRAY

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DECLARATION.

The work presented in this thesis is my personal work, carried out when I was in full-time employment, either as a veterinary practitioner in Kirkby Lonsdale, Cumbria (1984-86), or when appointed as Lecturer in the Department of Veterinary Clinical Science at the University of Liverpool since 1986.

This thesis has been composed by myself, under the supervision of Dr.C.D.Munro, from October 1986 to December 1988.

R.D.MURRAY.

Dedication.

To Marjorie, with grateful thanks
for her love and patience.

Contents.

SUMMARY.

Abortion episodes in cattle were investigated on 54 dairy farms in Cumbria, North Lancashire, and North Yorkshire from 1984 to 1986. From a breeding population of approximately 3600 dairy cows 149 abortions were investigated, representing an annual abortion rate of 2%, substantially higher than previously reported national estimates.

Visits were made by a Local Veterinary Inspector of the Ministry of Agriculture, Fisheries, and Food to each farm reporting an abortion, under the Brucellosis Order (England and Wales) 1978. The required samples were submitted to a Veterinary Investigation Centre (V.I.C), accompanied by form BS7. In addition, further samples were taken from each case where possible; placentome, fetal placenta, clotted blood (followed by a convalescent sample 28 days later), and fetus. All were examined in the practice laboratory.

Routine bacteriology was carried out on fetal stomach contents, lung, and placentome/placenta; cultured bacteria were identified. Fetal thoracic fluid and paired serum samples were sent to a V.I.C. for serological examination against Bovine Viral Diarrhoea virus (B.V.D), Infectious Bovine Rhino-tracheitis virus (I.B.R), and Leptospira hardjo infections.

Pathological studies were made on all fetal and placental material collected. Gross lesions were noted. Eyelid, heart, lungs, liver, and placental tissues were

routinely fixed in 10% formalin, sectioned, stained with Haematoxylin and Eosin, and examined.

Autolysis was present in 81 of 118 fetuses examined. Gross lesions were seen in 96 fetuses and 66 placental tissues. Except for hepatomegaly (3 cases), they were not considered to be diagnostically significance. Seven fetuses were mummified.

Immunoglobulins IgG and IgM were found in 35/129 fetuses, as well as titres to B.V.D (13/35), and L.hardjo (5/35). Positive titres to B.V.D, I.B.R, and L.hardjo were present in paired serum from 62 cows.

Histological lesions were demonstrated in tissue from 113 abortions; infection was associated with 57 episodes. In 9 cases which remained undiagnosed, consistent lesions of conjunctival hyperplasia with a coexisting placentitis (6/9) and amnionitis (2/9) were recorded, resembling experimental fetal infection with Ureaplasma diversum.

Myocarditis was associated with B.V.D (6 cases) and I.B.R (5 cases), which often coexisted with interstitial pneumonia (13 cases). Bronchial pneumonia occurred in 24 fetuses, associated with isolation of pure bacterial cultures from the lung. Erythroblastosis featured in 8 abortuses where L.hardjo infection was present.

Placentome was a diagnostically significant tissue; 18/40 presented a necrotic placentitis. Haemorrhagic lesions were found in 20, frequently linked to L.hardjo infection, but accompanied by hypoxic change.

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To my colleagues in the Veterinary practice, especially Ian Robinson and Bernard Carton, I owe a particular debt of gratitude. They were both extremely supportive to me in my task of compiling the cases and willingly made space for me to carry out the additional diagnostic work. I would also like to thank Mrs.S Robinson. BSc, for carrying out the practice laboratory procedures.

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We placed him
As a drop of seed
In a safe lodging
Firmly fixed;
Then fashioned we
The drop into a clot.
We developed
A lump; then
We developed out of that lump
Bones, and clothed the bones
With flesh;
Then we produced it
As another creature.
So blessed is God
The Best to Create.

from "The Qur'an verses.

Islam theology.

GENERAL INTRODUCTION

1.1. Definition

The English term abortion is derived from the Latin aboriri- to fail to be born. Black's Veterinary Dictionary (First Edition 1928) defined abortion as " the premature expulsion of the fetus or the contents of the pregnant uterus", a statement supported by the assumption that accident or disease was likely to be the cause. Deas (1981) has modified the term, defining it as the expulsion of a recognisable non-viable or dead fetus from the uterus.

Because the meaning of abortion has become too generalised, the Committee on Bovine Nomenclature attempted to re-define the terminology which described both embryonic and fetal death in pregnant animals. Hubbert (1971) published the results of these deliberations, and listed five separate headings under which losses during pregnancy could be described, namely:

i) embryonic mortality - deaths occurring within the period from conception until embryonic differentiation is complete ie. days 1-45.

ii) abortion - loss of the pregnancy occurring within the period from embryonic differentiation until term, the conceptus being incapable of independant life.

iii) premature delivery - the expulsion of the fetus before term but capable of independant life.

iv) stillbirth - the death of a fetus at term of normal pregnancy.

v) post-natal death - neo-natal death occurring immediately after birth and within the first 28 days of life, having survived the birth process.

1.2. Historical background.

John Knowlson (1834) described abortion in cattle as being an infectious disorder, transmitted by smell. It could spread throughout the farm steadings of whole villages, and was most likely to affect cattle from the sixth to eighteenth week of pregnancy. There was no treatment for the condition, but it could be prevented by rubbing tar onto the noses of cows at risk. Additional measures might include the burning of straddle-hay or wormwood inside the cow byre, or Spirits of Tar could be sprinkled liberally around the shippon. He derided some farmers who travelled to consult witch-doctors, in the hope of being told any plausible story concerning a neighbour who " might have cast an evil eye upon the petitioner's cattle". Upon receipt of his fee the witch-doctor could identify the culprit, describing the position of his house to that of the unfortunate victim.

Another early account is given in Clater's "Every Man his own Cattle Doctor". (published by Milner and Co, printers of other 1s.3d titles which included "The Drunkard's Wife" and "Little poems for Little People"). Published before 1885, it described abortion or "slink" as occurring towards the latter end of the year. It was associated with feeding cattle on fog or autumnal grass, located on low, marshy or fen ground.There was also a link with the presence and smell of carrion which could be found or exposed on grazing pastures. Treatment and prevention of "slink" demanded that cattle within affected herds be bled,

taking between two and four quarts of blood from each animal, and then drenched with potions containing Alum, Peruvian Bark, Aniseed, and treacle.

Tanner (1858) published the first account of a specific cause for bovine abortion, in which ergot of rye was found to initiate the premature expulsion of a calf. Affected animals were found to have grazed pasture that had "gone to seed" in September and become contaminated with ergot. These findings were presented as a definitive scientific explanation for abortion in the cow.

Frank gave the first account of infectious abortion in cattle in 1876, by introducing vaginal discharges from an aborted cow into the genital tract of a healthy, pregnant cow which subsequently aborted (Anon 1965).

Lupton (1885) must have been aware of this conflict of ideas, for in his monograph on "slink" he quoted both Clater and Tanner as references. He identified other possible causes; one was as a result of cattle "haunching" each other; another was that the condition was epizootic, provoking abortions in the fourth and fifth months of pregnancy.

Such references as these, found in the veterinary literature of Victorian Britain, seem to indicate that abortion in cattle was common. The disease even prompted a question being asked in the House of Commons in 1886, as to the necessity of bringing Abortion in Cattle under the Contagious Diseases (Animals) Act of 1867.

Against the background of a rapidly expanding dairy industry in Great Britain, the Highland and Agricultural Society of Scotland appointed a committee to investigate the then-known causes of abortion in 1886. Olver (1895) reported its findings and stated that;

i) abortion could be transmitted from one infected animal to other, healthy, cattle.

ii) abortion could be induced experimentally, following the inoculation of clean cattle with vaginal swabs taken from infected animals.

The mile-posts that describe how abortion was recognised as an epizootic or contagious disease may be summarised as below:

1897: Bang (1897) identified Contagious Abortion in cattle as a specific disease, caused by the organism Bacillus abortus.He described the gross lesions of necrotic placentitis, with its leather-like thickening of the fetal membranes and necrotic cotyledons, and isolated the organism.

Because of this work, subsequent veterinary research into bovine abortion was almost exclusively directed down this channel, for the next forty years.

1905: the Board of Agriculture and Fisheries set up a departmental committee to enquire into Contagious Abortion.

1909 and 1910: the enquiry described research into the incidence, transmission, and diagnostic methods

used. Recommendations were made which would require the notification of all suspected cases to the Board, isolation of infected cattle, and the restriction of cattle movements between affected and clean herds. The Serum Agglutination Test and Complement Fixation Test were developed to improve the diagnosis of the disease.

1913: the committee of enquiry reported the finding of a Vibrio-like organism associated with abortion outbreaks in Wales and Ireland.Theo Smith (1918) also identified a similar organism causing abortion in cattle in the United States. He named the organism Vibrio fetus.

1918: Alice Evans (1918) described B.abortus organism as being antigenically related to bacteria which caused Undulant Fever in man.

1920: Mayer and Shaw(1920) grouped the above organisms together as the genus Brucella.

Brucella abortus was thus identified as being a potential hazard to public health, and the need to control cattle abortion was now of paramount importance.

:Smith (1920) made the first isolation of Aspergillus fumigatus from an aborted bovine fetus.

1922: further restrictions on the sale and movement of aborted cattle were enforced under the Diseases of Animals Act (1910) Enzootic Abortion Order (1922).

1934: the economic losses suffered by the farming

industry as a result of B.abortus infection were beginning to be estimated. Buxton (1934) identified them as including the actual loss of the calf and the reduction in milk yield at the subsequent lactation.

Ainsworth (1934) had a wider perspective of the disease's affect upon livestock farming when he wrote, "this deplorable and constant waste of good breeding material cannot be allowed to continue under conditions which make special efforts to improve British stock-breeding more than ever necessary. Among the many problems requiring present attention are emphatically those which relate to the pathology of breeding."

1936: veterinary scientists working for the Ministry of Agriculture at Weybridge identified Trichomonas fetus as a cause of infertility and early abortion in cattle (Rees 1938).

1948: Absidia species were isolated from fungal abortions by Rollinson and Haq (1948), who observed that mycotic abortion was widespread although relatively uncommon. They outlined some of the pathological features of the condition: the presence of fungal hyphae in fetal stomach contents, a necrotic placentitis of the chorionic villi, and encrusting lesions on the skin of the fetus.

The financial implications of a growing abortion problem

in cattle were slowly being recognised. The Economic Advisory Council Committee on Cattle Diseases reported (Anon. 1934b) that of all herds surveyed, abortions occurring in 40% and within these, 9% of cattle aborted during the course of a calendar year. The same report (Anon 1934a) included results of a survey involving 2,830 herds containing 78,905 cattle. Of 17,247 animals culled from these herds, 23.8% were lost because of sterility and 3.0% as a result of abortion.The Ministry of Agriculture responded to this situation by introducing vaccination schemes against Contagious Abortion. Anti-abortion "A" vaccine was used with moderate success in non-pregnant cattle, but the anti-abortion "B" vaccine was quickly assessed as a failure in controlling an abortion storm in pregnant animals. The Ministry of Agriculture finally realised the limitations of these measures, when in 1942 the Control of Diseases of Dairy Cattle Scheme, otherwise known as the "panel" scheme, was launched. Its objectives were:

i) to improve control against a range of infectious diseases, including Contagious Abortion and infertility.

ii) to provide for the establishment of a free laboratory diagnostic and support service to Veterinary Surgeons and their farmer clients.

iii) to supply, free of charge, the new Strain 19 vaccine for calfhood vaccination against B.abortus infection.

(Kelly 1981).

The establishment of this unified laboratory diagnostic service, maintained by government out of the public purse, enabled efficient and accurate surveillance of animal diseases to be carried out.The National Survey of Diseases in Dairy Cattle,1957/8 (H.M.S.O.) showed the incidence of abortion to be 2.06% and that of stillbirths 1.78%, within the national herd. Less than 16% of these episodes were related to B.abortus infection as diagnosed by Veterinary Investigation Centres.

From within the Veterinary Investigation Service, Hignett (1951) reviewed some of the clinical aspects of V.fetus infection in cattle, and observed that if abortion occurred it was as a result of a placentitis without the fetus being infected. Lofthouse (1951) described the clinical features of a field outbreak of Vibrio infection in dairy cattle,but dismissed the findings of a concurrant Corynebacterium pyogenes infection present in the genital tracts of aborting cattle as of no significance. Ainsworth and Austwick (1955) and Austwick and Venn (1957) described their gross pathological findings in cases of mycotic abortion from which Aspergillus fumigatus was isolated. Specific caruncular or inter-caruncular lesions could not be consistently identified, but hyphae were visualised from direct smears off cotyledons which had dehisced. Aspergillus spp, Absidia spp, Polystictus spp, and Candida spp were also identified from fetal stomach contents. When cattle fetuses were examined at Weybridge and Bristol V.I. Centres

in 1957 as part of routine abortion investigations, 7.1% and 6.4% respectively of cases involved fungi. This was considered a cause of significant economic loss, comparable with that recorded for B.abortus and V.fetus infections.

Wilson (1959) recorded data compiled during investigations of 188 bovine abortion incidents submitted to Starcross V.I.Centre. Possible pathogens associated with each episode were identified in 21% of cases submitted and of these 65.7% related to B.abortus infections. In 1960-61 another National Survey of Cattle Diseases (H.M.S.O.) stated that 20-30% of British dairy herds had experienced abortions, but the incidence rate of B.abortus infection was only 2%; within 68% of these herds only one or two Brucella abortions were actually found. However, a serological screening of cattle in the United Kingdom revealed that 20% of the total cattle population gave a positive or inconclusive result to the Serum Agglutination Test (S.A.T.) for B.abortus. Bothwell (1963) estimated that 22% of dairy herds in England and Wales were infected with this organism, and that 32,000 cattle were producing infected milk. Only 50% of heifer calves reared annually in England were inoculated with Strain 19 vaccine and it appeared that control of the disease through this method alone was impossible. Of even more concern was the fact that about 100 human cases of Brucellosis had been reported by Public Health laboratories annually and that the true number of cases was likely to be greater. Such was the increased

awareness of the role that B.abortus played in zoonotic diseases of cattle, that it led to a demand from both the medical profession and the public for the disease to be eradicated.

In April 1967 the Ministry of Agriculture announced the setting up of a programme to eliminate Brucellosis, the Brucellosis (Accredited Herds) Scheme. By 1974 a British register of official Brucellosis-free herds was established and the first compulsory Eradication Area (in the New Forest) was declared in November 1976. All herds within the Area were to be brucellosis-free.

From the 1st November 1981 onwards, the scheme had progressed so that the whole of England and Wales had achieved full Accredited status, and only 0.14% of herds in the United Kingdom were ineligible for admission to the scheme's register.

1.3. The Brucellosis Eradication Scheme: its influence on abortion diagnosis in Veterinary Investigation Centres.

Reports issued by the Ministry of Agriculture, Fisheries and Foods Research and Diagnostic Laboratories (via the State Veterinary Journal) indicated just how great was the commitment of M.A.F.F. to the eradication of Brucellosis. V.I.Centre reports from the North Region (Anon. 1972) described the Brucellosis Scheme as "imposing a great burden of work" but they justified the effort in terms of a 2.5% positive B.abortus detection rate.

Meanwhile, from fetal material routinely submitted for diagnosis in other regions Salmonella dublin was isolated, as well as C.pyogenes (1.7% of cases) and fungi (2.0% of cases).

Routine investigation procedures carried out at Camarthen V.I.Centre were described by Hinton (1973). Enquiries into 258 abortions and full-term calvings were carried out. Except for B.abortus, organisms that were cultured routinely were not recorded and only contributed towards a negative result in tests for Brucellosis. A V.I.Centre report from Wales (Anon. 1975) described investigations into cases of mycotic and bacteria-related abortions, and concluded that a statistical analysis of the diagnoses achieved could not be taken as reliable since the specimens received by the laboratories were, to a large extent, governed by the statutory requirements of the Brucellosis Eradication Scheme. It was concluded that if

fetuses were to be submitted to V.I. Centres other diagnoses could be made.

From the South East Region, V.I.Centres reported (Anon. 1977a) that both S.dublin and Leptospira organisms were isolated in fetal material obtained from separate abortion outbreaks. The suggestion that Leptospires might be involved in the aetiology of abortion also came from the East and West Midlands Region (Anon. 1977b). These comments were made against a background of falling numbers of positive abortion diagnoses being recorded overall. A similar reduction in positive diagnostic rates was recorded by the West Midlands Region (Anon. 1977c).

The numbers of reported incidents of abortion have been recorded from M.A.F.F. Veterinary Investigation Data Analysis (V.I.D.A.) Annual Reports (1986) on fetopathies (Table 1).

TABLE 1.

M.A.F.F. Report on fetopathies (V.I.D.A.). Numbers of Bovine abortion submissions to V.I.Centres.

Year:	1975	1977	1979	1981	1983	1984	1985
No +ve diagnoses	3294	1813	1369	1532	1726	1402	1792
No -ve diagnoses	49608	37292	37488	33210	29919	28109	28865
TOTAL	52902	39105	38857	34742	31645	29511	30657

From the yearly reports of incidents summarised above, there has been a reduction of 42% in the number of abortion episodes recorded in 1985 compared to 1975. The positive diagnostic rate has altered only marginally, from 6% in 1975 to 5% in 1985.

1.4. Current approach to abortion diagnosis in the United Kingdom and abroad.

Present attitudes towards the diagnosis of abortion in cattle within the United Kingdom have been determined by a statement made by the Chief Veterinary Officer in November 1981 on completion of the Brucellosis Eradication programme, when he urged,

"that there be continuing vigilance in monitoring any abortions in cattle, which will be screened for Brucellosis by the Ministry's Veterinary Investigation staff."

The Diseases of Animals Act (1950) Brucellosis (England and Wales) Order 1978 requires the reporting by farmers of any cattle abortion occurring on their farms. It requires a Veterinary Surgeon to submit a single blood sample, milk, and vaginal swab from the aborting cow to a V.I.Centre, accompanied by a completed Form BS7. (Appendix 1).

Such samples are routinely screened by the laboratory for evidence of B.abortus infection, using cultural and serological techniques. Other possible infectious causes for the abortion may be identified, but the emphasis is directed towards control of Brucellosis. This diagnostic approach to investigate cases of abortion has effectively brought about the control of Brucellosis both in this country and abroad.

The approach to abortion diagnoses in the United States may be determined from work published by Hubbert and others (1973), where they found that in only three states was any clinico-pathological information available. Laboratory

diagnoses were mostly based upon microbial isolations from abortion material, and these were limited by the diagnostic capabilities of the laboratories themselves.The positive diagnostic rate was recorded as being 23-32% of total abortion submissions.

Kirkbride and others (1973) reviewed the incidence of bovine abortion in North America. The abortion rate was between 2-5%, still at the level prevalent in the 1960's before an eradication scheme for Brucellosis was finally introduced. B.abortus isolates accounted for less than 1% of the positive diagnoses, and this was cause for concern. With diagnostic rates of 20-25%, the authors called for a better understanding of the aetiology of abortion, in order to reduce the significant financial losses sustained by farmers.

Stubbings (1976) compared the differences in diagnostic methods used to investigate bovine abortions in the United Kingdom V.I.Centres with those in seven laboratories in the East and Central United States. The American investigators approach to the problem was based upon:

i) a pathological basis to all investigations.

ii) bacteriological and virological techniques used to support a diagnosis.

iii) fetal fluids used for microbial and serological identification of potential pathogens.

iv) placenta and/or caruncle used for pathological examination.

This was judged to be at variance with methods used by U.K. laboratories. He concluded that if a standard form of investigation could be adopted in abortion enquiries in this country, we could have a significant advantage over diagnosticians in the United States in terms of speed and accuracy of diagnosis, because of the United Kingdom's centralised Investigation service.

The submission of suitable field material to the laboratory has been recognised as a major problem for abortion investigators, both in this country and abroad. M.A.F.F. set up a project centred on Weybridge V.I.Centre (Anon. 1979), where Veterinary Surgeons and farmers were encouraged to submit aborted fetuses directly to the laboratory for investigation, but no results of that exercise have yet been published.

Dennis (1980 a,b) tried to tackle a similar problem in the United States. He placed the responsibility for successful abortion diagnoses squarely with the veterinary practitioner, who should supply the diagnostic laboratory with suitable field material; where possible, this meant the complete fetus and placenta. Minimum requirements were:

i) fetal stomach contents.

ii) fetal body-cavity fluids.

iii) fetal soft tissues - ice-packed.

iv) cotyledon.

v) blocks of cotyledon, fetal liver, lung, and intestine all placed in 10% formalin for histo-pathological

examination.

A similar attitude has been taken by Werdin (1979) and Kirkbride (1982). In addition, these workers have suggested that paired maternal serum samples, taken at the time of abortion and some four weeks later, could provide valuable additional information. Nusbaum and others (1978) and Evermann (1982) produced a field kit in which veterinary practitioners could collect suitable samples for an abortion investigation. Pathological material collected at post mortem from fetuses and their dams was placed in labelled containers, each filled with a suitable transport medium related to the intended laboratory examinations. This enabled a full range of diagnostic tests to be carried out on material which originated many miles away from the laboratory.

In Third World countries, most emphasis is placed on the submission of paired maternal serum samples to the investigating laboratory in order to make a diagnosis (Swanepoel 1975; Vardhan 1976; El- Naggar and others 1980), although submission of placenta and fresh fetal material is recognised as being most helpful.

Information gleaned from behind the Iron Curtain has suggested that investigative techniques for abortions are based upon a routine pathological examination, supported by bacteriology. Submission of fetal and placental tissues has formed the basis for laboratory investigations in East Germany (Bocklish and others 1981; Straub 1982),

Czechoslovakia (Venev 1974; Buchvarova 1982), and Hungary (Glavits and others 1982).

A multi-disciplinary approach to abortion diagnoses has been established in Australasia. During 1981, veterinary practitioners and Australian Department of Agriculture staff submitted a fresh fetus, placenta, and a single maternal blood sample from 24% of all abortion outbreaks investigated (Jerrett and others 1984), to enable microbiological, immunological, and histo-pathological studies to be pursued. Under conditions of management on the large, ranch-type cattle units, fetal and placental tissues were not always available and reliance had to be placed on uterine swabs and maternal blood samples (Davidson 1973). Samples required by New Zealand diagnosticians appeared to be fetal stomach contents, fixed fetal lung, and a maternal blood sample (Vickers and Brooks 1983). A common feature of both these countries' approach to this problem has been the increased use of serological techniques using maternal blood samples.

In France, Veterinary Departments have launched a national epidemiological study into the infectious causes of abortion in cattle other than Brucellosis, with priority being placed on possible zoonoses (Fedida and others 1980). In order to carry out this task, the National Laboratory for Bovine Diseases has been asked to recommend a standard investigative technique using micro-biological tests, tissue culture, histology, and electron microscopy.

1.5. Methods used for abortion diagnosis.

The investigation of abortion outbreaks has provided challenging problems to both veterinary practitioner and diagnostic laboratory on several accounts:

i) the aborted fetus is delivered days, or even weeks, after the cause of the abortion has affected either the dam or fetus.

ii) autolysis commonly masks fetal lesions so that comparison with experimentally produced fetopathies can only be made with caution.

iii) because of rapid autolysis which follows its death in utero, the fetus is not a suitable medium for pathogenic micro-organisms to grow in and multiply.

iv) case histories are usually incomplete.

The practicing veterinarian and laboratory diagnostician have key roles to play, in the supply of good quality specimens, and application of suitable diagnostic techniques that may provide the most relevant information, and the correlation of data (Kirkbride 1985).

Techniques employed in working towards a diagnosis are summarised below:

1) History taking: this is considered essential and should embrace the origins of the herd, its reproductive performance and breeding methods used, general herd health and vaccination programmes, together with information on nutrition and general management (Dennis 1980 b; Kirkbride 1982). This is

especially important when considering non-infectious causes of abortion, relating to nutrition (Griel and others 1969; Ray and others 1986), trace element deficiencies (Taylor and others 1979; Hidiroglou and Knipfel 1981), or possible toxicities (Macklin and Ribelin 1971).

2) Provision of suitable samples: however good the quality of submitted material might be, it is accepted that there are difficulties in arriving at a diagnosis when investigating single, sporadic abortions (Miller 1981; Kirkbride 1985). The minimum requirements for any enquiry are:

fresh/refrigerated fetal tissues:

abomasal or stomach contents.

lung and liver.

cotyledons.

formalin-fixed fetal tissues:

lung, liver, kidney, and other tissue displaying gross pathological change.

caruncle.

blood/serum:

maternal serum samples taken at the time of abortion, followed by one four weeks later.

The ideal material is considered to be the complete fetus and placenta, delivered to the laboratory as soon as possible after the abortion.

Swanepoel (1975) and Vardhan (1976) offered a different regime when investigating abortions under an extensive range system, where the only samples that could be obtained consistently were those of maternal blood and vaginal mucus. When Klastrup and Halliwell (1977) investigated infertility and abortion amongst range cattle in Malawi, they took additional samples of bull sheath washings.

3) Laboratory techniques: each laboratory employs its own, unique methods of diagnosis (Kirkbride 1985), but efforts have been made to standardise techniques in the areas of microbiology, immunology, histology (Kirkbride 1984), and the evaluation of relevant data (Deas 1981).

A summary is given in Tables 2 and 3 of the common microbiological methods in use: Tables 4, 5, and 6 describe some of the standard immunological techniques used by various authors in their own laboratories.

Direct light microscopy was the preferred technique for diagnosis of Trichomoniasis, carried out on fresh placenta or fetal abomasal contents (Kendrick 1975; Vardhan 1976; Klastrup and Halliwell 1977).

Direct virological cultures of fetal tissue have been carried out routinely in some laboratories, using bovine kidney or testis cell culture (Moojen and others 1983; Jerrett and others 1984).

In abortion cases where the history has indicated a likely non-infectious aetiology, biochemical studies have

TABLE 2:

Media used in isolation of bacteria associated with bovine abortion and incubated under aerobic conditions

Bacterial Isolate	Medium: Blood agar	Enrich tryp.	M/C	Plast.	Sabour.	Stuarts	Tryp.soy	Fletch. med.
B.lich'frmis	ad				a			
B. abortus	c	ci					i	
C.pyogenes	dif							
E. coli	f		dfh					
Leptospires						e		ef
Listeria	h	e						
Salmonella			ci					
Staphs	dfh							
Streps.	dfh							
Trichom's				bi				
Fungi					eghi			

Key to references:-

a) Ryan (1970)
b) Rodgers and others (1972)
c) Russell (1973)
d) Miller and Quinn (1975)
e) Roberts (1975)
f) Higgins and others (1981)
g) Neilan and others (1982)
h) Jerrett and others (1984)
i) Canant (1985)

Key to abbreviations:-

Bacteria :

B. lich'frmis - Bacillus licheniformis
Staphs - Staphylococci spp.
Streps - Streptococci spp.
Trichs - Trichomonas spp.

Media :

Enrich tryp - Enriched tryptose
M/C - MacConkey's
Plast - Plastridge's medium
Sabour - Sabouraud's medium
Tryp. soy - Trypticase soy
Fletch - Fletcher's medium

TABLE 3

Media used in isolation of bacteria associated with bovine abortion, incubated in 5 or 10% carbon dioxide.

Bacterial isolate	Brucella agar	Blood agar	Enrich trypt	Farrells med.	Fletch. med.	Myco agar	Tryp	Urea agar
	Medium							
B.lich'fmis		aj						
B.abortus	e		ig	h			dg	
C.pyogenes		dgi	di					
Camp'bctr		dgi.						
Listeria		di	dgi					
Mycoplsma						e		
Salmonella		b						
Ureaplasma								ecf
Leptospira					g			

Key to references:-

a) Ryan (1970)
b) Russell (1973)
c) Miller and Quinn (1975)
d) Kirkbride (1975a)
e) Higgins and others (1981)
f) Ruhnke and others (1984)
g) Kirkbride (1984a)
h) Jerrett and others (1984)
i) Canant (1985)
j) Mitchell and Barton (1986)

Key to abbreviations :-

Enrich trypt - Enriched tryptose
Med - Medium
Fletch. med - Fletcher's medium
Camp'bctr - Campylobacter
Tryp - Trypticase
Urea agar - Ureaplasma agar
Myco agar - Mycoplasma agar

TABLE 4

Serological techniques using maternal sera (single and paired) as aids to diagnosis in bovine abortion.

TEST	Aden	B. abort	BVD	Camp	Chlam	IBR	Lepto	M. wolfii	Parvo	PI_3
Complement fixation	mp					jf		m		
Enzyme linked					d					
Haemagglut inhibition			k						i	a
Micro agglut'n							ghc mop			
Macro agglut'n				ed lo						
Rose ben		ep								
Serum Neutralis'n	f		hn op			ob mp				a

Reference key:-

a) Dunne and others (1973)
b) Kirkbride (1975a)
c) Roberts (1975)
d) Vardhan (1976)
e) Klastrupp (1977)
f) Krpata and others (1978)
g) Mackintosh & Marshall (1980)
h) Miller (1980a)
i) Bodine (1981)
j) Storz and Whitman (1981)
k) Moojen and others (1983)
l) Bryner (1984)
m) Jerrett and others (1984)
n) Kendrick (1984)
o) Kirkbride (1984b)
p) Canant (1985)

Key to abbreviations:-

Aden - Adenovirus
B.abort - Brucella abortus
BVD - Bovine Viral Diarrhoea virus
Camp - Campylobacter fetus vars
Chlam - Chlamydia
IBR - Infectious Bovine Rhino-trachitis
Lepto - Leptospira hardjo
M.wolfii - Mortierella wolfii
Parvo - Parvovirus
PI3 - Para-influenza 3 virus
Rose Ben - Rose Bengal plate agglutination test

TABLE 5:

Serological techniques using fetal fluids or serum as aids in abortion diagnosis.

TEST	IgM/IgG	Chlamydia	Enterovirus	BVD	Lepto.	Pl_3
Agar-gel diffusion	bcd					
Enzyme linked		i				
Haemagglut'n inhibition			a			a
Immuno electrophoresis	h					
Micro agglutination					fg	
Serum neutralisation				ceg		

Key to references:-

a) Dunne and others (1973)
b) Ellis and others (1978)
c) Miller (1980a)
d) Ohmann (1981)
e) Kendrick (1984)
f) Kirkbride (1984b)
g) Canant (1985)
h) Jerrett and others (1984)
i) Storz (1984)

Key to abbreviations:-

BVD - Bovine Viral Diarrhoea virus
Lepto - Leptospira hardjo
Pl_3 - Para-influenza 3 virus

TABLE 6

Immunological techniques using fetal tissue sections as an aid in abortion diagnosis.

DIAGNOSIS	FLUORESCENT ANTIBODY TEST DIRECT	INDIRECT
Adenovirus		c
BVD	g	c
C pyogenes	h	
Coronavirus		c
Campylobacter	ah	
Chlamydia	k	g
IBR	adgl	c
Listeria	bdj	
Leptospira	aei	
Parvovirus		f
PI_3		c
Salmonella	l	

Key to references:-

a) Roberts (1975)
b) Smith (1975)
c) Miller and Wilkie (1979)
d) Miller (1980a)
e) Ellis (1980)
f) Bodine and others (1981)
g) Kendrick (1984)
h) Bryner (1984)
i) Kirkbride (1984b)
j) Smith and Dennis (1984)
k) Storz (1984)
l) Canant (1985)

been routinely employed using maternal serum samples. Tests used include those for serum protein, haemoglobin, packed cell volume, total bilirubin, aspartate transaminase, and lactate dehydrogenase in an investigation for aflaxtoxicosis (Ray and others 1986). In an investigation of abortion thought to have been caused by pesticide contamination of cattle feedstuffs, fetal peritoneal fat, liver, and kidney were examined for toxic residues (Macklin and Ribelin 1971). In localities where selenium deficiency is a known problem, quantitative fetal liver assays have been developed (Taylor and others 1979).

1.6. The pathological examination of abortion material: its role and relevance.

Fetal and placental lesions found in both clinical and experimental infections associated with abortion episodes in cattle have been described, particularly those in cases of B.abortus (Jubb and others 1985), A.fumigatus (Hill and others 1971), L.monocytogenes (Osebold and others 1960), and S.dublin (Russell 1973) infections. There are few reports of field investigations into cattle abortions in the United Kingdom where any importance has been attached to the gross or histopathological lesions found, and little comment as to their significance. Thus, the veterinary practitioner has not been encouraged to submit abortion material for a pathological examination, neither have laboratories expended time or money on this aspect of diagnosis.

This is in marked contrast to the diagnostic approach to abortion in sheep, where pathological lesions found in the placenta and fetus associated with toxoplasmosis and enzootic abortion have been considered almost pathognomonic. V.I.Centres have positively encouraged the submission of this material for diagnostic purposes, to very good effect.

In human medicine, doctors have previously held the belief that a pathological examination of the placenta was too complicated, and diagnostically unrewarding. This may be a reflection of an incomplete understanding of many aspects of placental pathology, or because of the on-going

controversy concerning non-infectious causes of placental disease and abortion (Fox and Langley 1971). As in veterinary medicine, much of the material produced after human abortions is usually fragmentary and incomplete. Nearly half of all fetuses examined in one survey were macerated to some degree (Paverstein 1979).

Fetal autolysis has been a constant problem in all pathological investigations of abortions. In cattle, some infections quickly cause fetal death in utero but some delay may occur before the fetus is expelled. With Infectious Bovine Rhino-tracheitis Virus (I.B.R) abortions, this period may be up to seven days, and with S.dublin expulsion of the fetus has been known to occur up to eleven days after its death (Hall and Jones 1976).In other fetal infections, such as B.licheniformis and C.pyogenes, fetuses have shown evidence of having been dead in utero "for some time" (Ryan 1970; Deas 1981)

Dennis (1980 b) found that autolysis was the most common finding in aborted fetuses, the degree being dependent upon the time and cause of death. Gross signs included:

i) corneal opacity.

ii) oedema in subcutis and serous cavities.

iii) rapid liquefaction of the renal cortex.

iv) pale, soft liver.

v) cloudy, yellow/red abomasal contents.

It is only after intra-uterine death that any oedematous fluid in the fetus becomes blood-tinged, usually within 12-

36 hours. All tissues may develop a uniform reddish appearance as a result of haemoglobin staining. These changes frequently hide lesions which could be of diagnostic importance (Kirkbride 1982).

It is this particular factor which may account for the opinion of many veterinary pathologists that routine post mortem examinations of aborted fetuses are of limited value in achieving a diagnosis when investigating abortion outbreaks.

1.7. Objective of this study.

It is the purpose of this thesis to assess the value of a gross and histo-pathological examination of fetal and placental tissues in the diagnosis of bovine abortion, and to record those lesions found in a routine examination of abortion material collected from field cases at the time of a routine abortion enquiry. A diagnosis will be made on the basis of the observed lesions, supported by micro-biological and serological laboratory examinations in cases of known infectious or non-infectious aetiology. Lesions which are consistently observed but have no corroborative laboratory evidence to support a diagnosis will be high-lighted, and their significance discussed.

LITERATURE REVIEW.

2.1. The significance of fetal and placental lesions in the diagnosis of bovine abortions.

Pathological changes in fetal or placental tissues may act as a guide towards a diagnosis in cases of infectious abortion which is subsequently confirmed by isolation and identification of an agent (Dennis 1969). Many Veterinary Investigation laboratories have accepted this. Most diagnoses of infectious abortions in cattle are based upon a correlation between both gross and histological lesions presented in a fetus or placenta, and identification of likely infectious agents by direct methods. Probable abortive agents may not be considered significant unless appropriate ante-mortem lesions, such as a suppurative placentitis, can be demonstrated microscopically (Werdin 1979; Canant 1985).

With technological advances constantly being made in diagnostic methods for bacterial and viral infections of animals, criteria for diagnosing disease have changed . In his laboratory, Dennis (1980a) based abortion diagnoses on the evaluation of the herd's previous history, gross and histological fetal or placental lesions, and their correlation with possible infectious agents identified by direct or indirect means. Placenta was the tissue of greatest diagnostic value because placentitis was the lesion common to all cases of infectious abortion.

Many species of bacteria are widespread and their mere presence in abortion material cannot be considered as absolute evidence of pathogenicity. Problems may arise with any diagnosis if non-pathogenic, slightly pathogenic, or pathogenic organisms known to cause disease in cattle other than abortion, are isolated without a description of associated histological lesions in the aborted material under investigation (Kirkbride 1984).

Alternatively, Jerrett and others (1984) have argued that undiagnosed infections may be associated with abortion episodes where specific diagnostic inflammatory lesions in the fetus or placenta are recognised histopathologically, without any concurrent evidence of an aetiological agent. If this statement is valid, evidence that infectious agents play a major role in cattle abortion outbreaks may be significantly strengthened. Miller and Quinn (1975) found lesions in 48 aborted fetuses out of 50 and detected antibody ie.IgG and IgM, in 45.8% of them. Kirkbride and others (1977) examined histologically 160 abortuses and found lesions of varying degrees of significance in 114; both inflammatory and necrotic changes were classified as lesions. Of the total numbers examined, 62.8% of fetuses contained immuno-globulins IgG and IgM in concentrations of more than 20mg/ml. Higgins and others (1981) recorded significant pathological changes in 60% of bovine abortion material examined histologically, and Ohmann (1981) described lesions in 88.2% of abortuses examined. These

observations suggest that many undiagnosed causes of abortion may have an infectious aetiology, if these pathological findings in fetus and placenta are accepted.

This point can be illustrated by a clinical condition well recognised both epidemiologically and pathologically, but where the precise aetiology was unknown, such as epizootic bovine abortion (E.B.A.). The disease colloquially known as "foot-hill abortion", occurs in the grazing lands of the Sierra Nevada and coastal hills of California. Cattle frequently abort in late pregnancy or produce weakly calves at full-term. The disease is considered to be an infection, since experimental transmission of the disease via the tick Ornithodorus coriaceus has been successfully accomplished (Kimsey and others 1983). Whilst the infectious agent was unknown, the pathology of the condition was universally acknowledged; affected fetuses exhibited a lymphocyte blastogenesis, with proliferation of lymphocytes and macrophages particularly in lymph nodes. Macrophage infiltration in the portal zones of the liver and alveolar walls of the lungs led to formation of focal granulomas and sub-acute perivasculitis. With established infections of some age, extensive loss of thymic tissue occurred. These changes were considered to be unique to this condition (Kennedy and others 1983). Lately, Osebold and others (1986) have compared the lesions of E.B.A. with those presented in the condition of congenital treponematosis and suggested that this pathological picture could arise from persisting

spirochetosis.

The significance of a histopathological investigation into abortion outbreaks where non-infectious causes are suspected has been outlined by Nusbaum and others (1978), who have suggested a systematic approach to diagnosis. An initial screening of fetuses using serological techniques would eliminate the common infectious diseases, so that the more intractable cases could be subjected to a broader investigation which would include histopathology. Such an approach was described by Taylor and others (1979), who investigated cases of abortion and reproductive failure in cattle of Western Canada. Their initial histological findings were those of vascular damage which produced degeneration and necrosis in target organs such as brain, skeletal and cardiac muscle, liver and kidney; selenium deficiency was diagnosed, confirmed using biochemical techniques, and suggestions were made as to the possible role of prostaglandins in such abortion outbreaks. The vascular changes observed in this study were similar to those found in cases of avian encephalomalacia, porcine hepatosis dietetica, and white muscle disease, conditions all characterised by abnormalities of the blood-clotting cascade mechanism and formation of thrombi.

Histopathological examination has played a major role in investigations which related thyroid gland enlargement to perinatal mortality in cattle. Shafie and Mashaly (1974) have described the normal developmental anatomy of the

thyroid gland in bovine fetuses. Biolatti and others (1982) found histological abnormalities of varying severity in thyroid glands of 89 fetuses, ranging from follicular hypertrophy to intra-follicular papillomata proliferation with the absence of colloid. Such goitrous changes that were found in these glands may be ascribed to either a lack of dietary iodine or a high intake of foodstuffs containing goitrogenic compounds (McAllum 1984).

Fetal pathological examinations played an important role in the Kansas Genetic Disease Programme (Leipold 1980), where congenital defects of cattle associated with abortion, stillbirth, and early neonatal death were investigated in detail. Accurate diagnosis of conditions such as arthrogryposis,congenital hydrocephalus, mannosidosis, and polydactyly were made so as to effectively control and prevent calf losses related to genetic defects of known aetiology. Changes in management and improvements to breeding programmes were then effectively introduced.

2.2. The infectious causes and lesions associated with experimental and field cases of abortion in cattle.

Brucella abortus.

B.abortus infection is usually associated with infectious abortion in cattle, but cross-infections do occur in other mammals. Shaw (1976a;b) described a naturally active field infection of pregnant ewes running in a commercial hill flock, and Brucellosis is a well-documented zoonotic disease of man (Bevan 1921).

The organism can localise and persist in many organs but most readily infects the genitalia, placenta, and mammary gland of cattle. The source of infection is usually an aborted fetus, its placenta, or contaminated uterine discharges. The organism enters the body through intact mucous membranes or broken skin. Infection may persist in cattle indefinitely, usually in regional lymph nodes, and then spread by the blood to other soft tissues where it becomes localised.

B.abortus has an affinity for the pregnant cow's endometrium and if the infection is severe, abortion is the likely outcome. This most commonly occurs in the seventh or eighth months of pregnancy, after which the organism is cleared from the uterus within a matter of weeks (Jubb and others 1985).

Gross pathological lesions in the placenta have been described as a necrotic placentitis, the affected cotyledons

being yellow/grey in colour, soft, and necrotic. Covering them and extending over some inter-cotyledonary portions of the placenta, a brown, sticky, viscous exudate may be found containing yellow floccules of debris. All cotyledons are not uniformly affected (Smart 1975).

The fetus may be oedematous with sero-sanguinous fluid present in peritoneal and thoracic cavities. Abomasal contents are turbid, pale yellow in colour, and contain cellular debris. Gross evidence of a broncho-pneumonia in the lungs can be variable but when present, oedema and fibrinous tags are evident (Kirkbride 1985).

Jubb and others (1985) described the histological lesion found in placenta and fetus. The placental stroma was oedematous with a mixed inflammatory cell infiltration, and chorionic epithelial cells were stuffed with bacteria. The most severe inflammatory reaction was at the base of the cotyledonary villi, where many giant cells were necrotic. Inflammatory exudate could accumulate between the maternal and fetal epithelium, particularly in the placental arcades. Maternal portions of the placentome were seldom affected except for the expanded ends of the maternal villi when they became engulfed by exudate from fetal villi. Beneath the tips of these villi a moderate leucocyte infiltration could be found with fibrosis in the core of the villi.

In the fetus, microscopic lesions of broncho-pneumonia and scattered foci of bronchitis may be found with a heavy

mononuclear and polymorphonuclear inflammatory cell infiltration of the stroma, and air-ways that were stuffed with necrotic debris and inflammatory cells. The inter-lobular septa may become oedematous, with lymphatics infiltrated with leucocytes. Other variable lesions can include a necrotising arteritis, especially of pulmonary vessels, and focal areas of necrosis and granulomata with giant cell formation in liver, spleen, and lymph nodes.

Bacteria of the genus Bacillus.

The first field cases of abortion associated with Bacillus infection were recorded in Tasmania (Mason and Munday 1968), and from North America (Wohlgemuth and others 1972a). Both groups of workers, whilst suggesting that these organisms were of low pathogenicity, were aware that all bacteria of this genus produce a necrotizing toxin. Bacillus licheniformis was first associated with bovine abortion outbreaks in the United Kingdom by Ryan (1970).

Pregnant heifers have aborted following experimental infection with Bacillus ceres (Wohlgemuth and others 1972b). The important lesions were a placentitis and autolysis of the fetus. Placental examination revealed inter-cotyledonary thickening, adventitious placentation, and cotyledonary necrosis in places where the elements of the placentomes had not separated after birth. A necrotic placentitis was described histologically, with inflammation and necrosis of

the arcade zone of placentomes. There are no accounts of lesions following experimental B.licheniformis infection.

Field cases of abortions related to B.ceres have been described by Schuh and Weinstock (1985). Placental cotyledons and inter-caruncular areas were soft and light-brown in colour. Microscopically, the lesions were a moderate to severe diffuse necrotising suppurative placentitis with an accompanying vasculitis. Fetal lesions were those of a mild diffuse suppurative interstitial pneumonia.

Three field cases related to B.licheniformis infection have been described by Mitchell and Barton (1986). They were characterised by a necrotic placentitis with foci of chorio-allantoic necrosis involving both a neutrophilic and eosinophilic cellular infiltration. Chorionic vasculitis was a prominent feature, with some thrombosis. In the fetus, a mild to moderate autolysis was present, with fibrin strands in pleural and peritoneal cavities. The lungs were consolidated and pus could be expressed from air-ways. Histologically, the lung lesions were a suppurative pneumonia with exudate and inflammatory cells present in air-ways, together with an inflammatory cell infiltration of alveolar septae. There were also accumulations of mixed inflammatory cells in peri-bronchial areas and inter-lobular septae where oedema was also located. Other microscopic lesions described were a fibrinous pericarditis, focal hepatitis with inflammatory cell infiltration of the peri-acinar portions of the lobules, and a patchy suppurative encephalitis with

associated neutrophil infiltration. These findings were similar to those described by Mason and Munday (1968), who recorded fetal lesions in abortions where an unspecified Bacillus organism was implicated.

Bacteria of the genus Campylobacter.

Campylobacter fetus var. venerealis infection of cattle is commonly associated with herd infertility and repeat-breeder cows (Park and others 1962), and it has been diagnosed with increasing frequency in the United Kingdom since the mid-1970s (Deas 1981). The organism has also been implicated in sporadic abortion outbreaks in North America (Whitford and others 1977), although C.fetus var. fetus is the strain more commonly isolated from field cases of abortion.

Experimental inoculation of viable cultures of C.fetus var. venerealis into the uteri of cattle during the middle and third trimesters of pregnancy has been shown to cause abortion (Osburn and others 1969). Fetal death occurred several days before abortion occurred. Gross lesions were seen in the placenta, consisting of flaccid, tawny to grey-white cotyledons containing multiple necrotic yellow/grey foci of necrosis. The inter-cotyledonary chorio-allantois was also stained red/brown, with numerous small, yellow/grey foci coalescing to form larger plaques on the chorionic surface.

Microscopic changes were found in cotyledonary villi,

consisting of villous necrosis and necrotic detritus on the chorionic surfaces. Oedema of the inter-cotyledonary mesenchyme was obvious, as was ulceration, associated fibrin and mixed inflammatory cell accumulations, and vasculitis in medium-sized arterioles. No related fetal lesions were described, save those of extensive autolytic change.

Field cases of bovine abortion associated with Campylobacter infection have produced placental lesions essentially similar to those found with experimental infections (Deas 1981). However, fetal lesions were significantly different as Bryner (1984) has indicated. In his series of cases, autolysis was not at all common; indeed, many fetuses were found to be born alive although dehydration, fibrinous peritonitis, pleuritis or pericarditis were frequently seen on post mortem. Fetal livers showed evidence of increased friability as rupture was a common gross finding, with associated massive peritoneal haemorrhage.

Bryner (1984) has recorded more diverse fetal microscopic lesions, ranging from pneumonitis to suppurative broncho-pneumonia in lungs and mild bile duct hyperplasia to hepato-cellular swelling and vacuolation in the liver. Myocardial vacuolation has also been described by Whitford and others (1977).

Organisms of the genus Chlamydia.

Much confusion has arisen concerning the role of Chlamydia species in bovine abortions. Storz and others (1960) presumed that the condition of Enzootic Bovine Abortion (E.B.A) was caused by Chlamydial infection of cattle, an opinion based upon pathological evidence alone. Several workers (Sadowski and Truszczynski 1972; Akkermans and Dinkla 1974) have assumed that their own observations of fetal lesions in abortions genuinely associated with Chlamydia have confirmed Storz's original findings. Later, Storz and Whitman (1981) described the lesions found in experimentally induced Chlamydial abortions, but they did not relate their findings directly with E.B.A. Kennedy and others (1983) could not recover Chlamydia from any field outbreaks of E.B.A. neither could they isolate the organism during extensive transmission experiments.

Page and Smith (1974) inoculated a human abortifacient strain of Chlamydia into pregnant cattle which caused them to abort and produced a marked placentitis. Observed lesions consisted of a thick, yellow/grey exudate over-lying necrotic chorionic epithelium with localised areas of oedema in the inter-cotyledonary chorio-allantois. A progressive invasion of inflammatory areas extended into basal and arcade zones of affected cotyledons, whilst others were completely unaffected. Microscopic lesions were variable. Inter-cotyledonary lesions ranged from oedema to a fibrino-purulent exudate composed of necrotic epithelial and other

cell debris. Within placentomes, there was an extensive inflammatory involvement; inter-cryptic haemorrhage and a dissecting necrosis with exudate separated maternal epithelium from fetal trophoblast.

Storz and Whitman (1981) found that experimental placental lesions varied with the stage of gestation and duration of the infection. If infection and abortion occurred before the sixth month of gestation, the entire placenta was discoloured, and extensive oedema was present in the inter-cotyledonary areas. With later abortions only small portions of the placenta were abnormal, the chorion being of a tough, leathery consistency and the surface adjacent to the endometrial epithelium a reddish/white opaque colour. The margins of cotyledons bordering these affected areas contained small foci of necrosis, with a bulging oedema surrounding them on the fetal side of the membrane. Occasionally, Chlamydial cytoplasmic inclusion bodies were demonstrated in endometrial epithelial cells and trophoblast.

The aborted fetuses examined by Page and Smith (1974) were generally under-weight for age. Some fetal livers were discoloured, enlarged, and coated with fibrin. Ascites was an occasional finding. Histological lesions in these fetuses were minimal; only a mild peri-vasculitis and some scattered multifocal areas of mononuclear inflammatory cell infiltration were located in the liver. Storz and Whitman (1981) were unable to demonstrate any significant lesions in

calves born three to four months after maternal exposure to infection. In fetuses aborted before the sixth month of gestation, necrotising foci surrounded by inflammatory cells were observed in liver, spleen, kidney, heart muscle, lungs, adrenal glands, and in the central nervous system. Hepatic lesions were mid-zonal and consisted of necrotic hepatocytes with small accumulations of monocytes and neutrophils; any phlebitis involved veins adjacent to these inflammatory foci. Vasculitis was a common finding in other soft tissues.

There is no report in the literature which enables a direct comparison to be made between these experimental fetal and placental lesions and those which can be found with field cases of abortion in cattle, associated with Chlamydia infection.

Corynebacterium pyogenes.

Hinton (1972) has reviewed the role played by C.pyogenes in the aetiology of abortion in cattle, and concluded that it was of minor importance. He did admit that, on certain farms, the organism could be associated with significant fetal death and infertility but that this was sporadic in the main. Smith and others (1971) and Bucharova (1982) have described C.pyogenes as a frequent cause of bacterial abortion in cattle. Smith (1984) stated that this organism is a common inhabitant of normal conjunctival, vaginal, and preputial mucosae of the bovine, and that it is seldom found as a contaminant or commensal

organism in tissues of aborted fetuses or their placentas. Its presence in such material was usually significant.

Smith and others (1971) investigated the effects of experimental C.pyogenes infection on the placentome of ruminants, taking the sheep as a model. The most consistent gross feature was a placentitis, with oedema of the chorio-allantois and petechiated areas located near the umbilical chord. When retained fetal membranes (R.F.M) were a clinical feature of these abortions, the placentomes were uniformally oedematous. Opposing surfaces of caruncle and cotyledon could be red, brown, or brownish/green in colour. Microscopic lesions were present in placentas recovered from infected animals that were pregnant from 90 days onwards. Haemorrhages were found in septa of uterine caruncles, with bacteria most prominent in the hilar region at the periphery of the chorionic villi and within dilated maternal capillaries at the septal tips. In early cases of infection, an inflammatory response was virtually absent. As the infection became established in later pregnancy, micro-abscesses appeared within the deeper stroma of the caruncles which later formed a confluent band of inflammatory tissue near the basal areas of the placentome. In aborting sheep, this zone was located at the cotyledonary surface. Within uterine glands adjacent to areas of desquamated cellular debris many neutrophils were found, many of them loaded with phagocytosed bacteria and resting on endometrial epithelium. Occasional cotyledonary villi were calcified. No inter-

cotyledonary lesions were encountered.

In these experimental cases, no fetal lesions were described other than those of autolysis, which varied only by degree. Bacteria could be identified in the air-ways of fetal lungs but associated inflammatory changes were often completely absent.

Numerous gross lesions were reported in sheep abortuses following C.pyogenes infection during pregnancy (Smith 1984). In fetuses aged up to 4 months, white/yellow foci were found in lungs; in older lambs, a fibrinous pericarditis, pleuritis, or peritonitis was descried together with a suppurative broncho-pneumonia.

Schiefer and others (1974) have detailed the histological lesions associated with experimental fetal inoculation of C.pyogenes. A purulent broncho-pneumonia with inflammatory cell exudate and bacteria within the air-ways could expand to a necrotising fibrinous pneumonia. Where the exudate was less dominant, a peri-bronchial and peri-vascular accumulation of mononuclear cells was evident. Liver lesions of inflammation and peri-vasculitis, increased numbers of splenic megakaryocytes, and the presence of oxalate crystals in kidneys were occasionally observed.

Bacteria of the genus Diplococcus.

Diplococcus lanceolatus has been isolated from aborted bovine fetuses and their placentas by Angelov (1974). He described the associated pathological changes as being

similar to those found in cases of streptococcal abortions. No gross lesions were found in the fetuses, but the placenta presented evidence of oedema, haemorrhage, and focal yellow or green infiltrative lesions that were together described histologically as a suppurative necrotic placentitis.

Microscopic lesions in these fetuses were those of interstitial pneumonia and a widespread, mixed inflammatory cell infiltration in the liver.

Bacteria of the genus Haemophilus.

Haemophilus somnis has been demonstrated as the aetiological agent in cases of bovine thrombo-embolic meningo-encephalitis, and is an important isolate in the differential diagnosis of diseases of the central nervous system in feed-lot cattle of North America (van Dreumal and others 1970; Miller and others 1983a). The organism has a world-wide distribution and would appear to be an important pathogen of the female bovine reproductive tract (Miller 1980b). It has occasionally been isolated in association with abortion episodes (Chladeck 1975; van Dreumal and Kirkstead 1975).

Experimental infusion of strains of H.somnis into the uteri of pregnant cattle via the cervix failed to induce abortion during late pregnancy (Miller and Barnum 1983), but intra-amniotic inoculation of six pregnant cattle produced clinical signs of bacteraemia and pyrexia in all animals and induced abortions (Miller and others 1983b).

Macroscopic lesions were only found in the placenta,

and consisted of a peripheral thickening of cotyledons and failure of placental membranes to separate from their placentomes.

Microscopic lesions were located in both placenta and fetus. Severe necrosis with a neutrophil infiltration was observed in the chorio-allantoic villi, together with widespread arteritis and mononuclear cell infiltration of the tunica of affected blood-vessels which were occasionally thrombosed. A mononuclear cell infiltration within the interstitial tissue of the chorio-allantois was commonly encountered. On occasion, some placentomes contained areas of coagulative necrosis with bacteria located in trophoblast cells. In the amnion there was a mixed inflammatory cell reaction present, with fibrinous exudate and bacteria.

Fetal lesions were consistently recorded. The sub-conjunctival tissue contained a mild mononuclear cell infiltration which included occasional eosinophils, as well as clumps of bacteria within the lamina. In fetal lung, the air-ways and alveolar ducts were packed with mononuclear cells and bacteria. Inter-lobular lymphatics could be distended with fibrinous exudate. From time to time, bacteria could be found in the blood vessels of other organs, particularly the liver.

van Dreumal and Kierstead (1975) gave an account of the lesions that were discovered during investigations of field cases of abortion and were related to a diagnosis of H.somnis infection. Whilst there was good preservation of

the fetus, cotyledons were oedematous and necrotic as were areas of inter-cotyledonary chorio-allantois where there was also some haemorrhage. Microscopic lesions in the placental stroma were those of oedema and a moderate mononuclear cell infiltration. Many blood vessels were thrombosed. Villous necrosis was marked and bacterial clumps were seen within the necrotic debris.

Fetal lesions were descrived: in the heart an interstitial myocarditis involved mostly mononuclear cells with some myocytolysis evident; in the liver there was a mild peri-portal mononuclear cell infiltration; hyaline thrombi were common in the glomerular capillaries of the kidney and there was focal tubular necrosis in the cortex; lung lesions were essentially similar to those recorded in the experimentally induced abortions.

Bacteria of the genus Leptospira.

There is ample circumstantial evidence to associate Leptospira interrogans var.hardjo with abortion in cattle, both in the United Kingdom (Ellis and others 1982; Hathaway and Little 1983a; Ellis and others 1985), in Australia (Hoare and Claxton 1972; Slee and others 1983), and in Canada (Kingscote and Wilson 1986). The diagnosis has been made either by demonstration of fetal infection and an immuno-logical response to it using a combination of laboratory tests involving fetal serology, immuno-fluorescence, and bacterial isolation (Ellis and others

1982), or from paired serum samples taken from the aborting cow (Kirkbride 1984b).

Pathological methods used to confirm such a diagnosis have been based upon demonstration of the organism by staining tissue sections of liver and kidney, using a silver-impregnation technique (Drury and Wallington 1967) or fluorescent antibody (Ellis 1980).

Descriptions of placental and fetal lesions associated with field outbreaks of L.hardjo abortions are not consistent. Dennis (1980a) described the placental lesions as a diffuse placentitis, cotyledons being a light-tan to yellow colour, atonic, avascular, and uniformally affected. The inter-cotyledonary placenta could be diffusely oedematous, covered with a yellow/brown gelatinous fluid. Dennis (1969) had earlier suggested that this uniform placental lesion was typical for this infection. Kingscote and Wilson (1986) have reported cases presenting non-specific mineralisation in four out of six placentas recovered during investigation of a single large outbreak. Other workers have been unable to corroborate these findings (Slee and others 1983; Jerrett and others 1984; Kirkbride 1985).

There are several descriptions given of fetal lesions. Slee and others (1983) found histopathological changes of a mild focal interstitial nephritis in two out of five abortuses examined. Ellis and others (1976a) found severe focal vascular lesions in many soft tissue organs from two

aborted fetuses, as well as centri-lobular degeneration and necrosis in hepatic lobules. Kirkbride (1985) found pneumonic lesions in some fetuses.

Bacteria of the genus Listeria.

Listeria monocytogenes has been cultured from aborted bovine fetuses in many parts of the world (Gray 1957). Experimental infection of pregnant cattle using field strains of L.monocytogenes obtained from abortion material has caused fetal death and subsequent abortion (Osebold and others 1960). A most comprehensive description of feto-placental lesions (see Tables 7 and 8) has been given after

TABLE 7

Placental lesions associated with abortion in pregnant ewes after experimental infection with L. monocytogenes.

Cotyledon	Fetal lesions: Inter-cotyledonary zone	Maternal lesions: caruncle
Macroscopic appearance: enlarged, usually generalised	Loss of translucency; thickened; hyperaemic; oedematous, with necrosis. Rather friable. Red to red-brown exudate.	Enlarged, with some loss of concavity. A dull meaty, nodular appearance. On cut surface, areas of hyperaemia and necrosis, extending from the hilus to stratum compactum
Microscopic appearance: Neutrophil infiltration in the villous mesenchyme and chorionic epithelium, together with calcification; bacteria present.	Autolysis common; oedema and sloughing of chorionic epithelium. Neutrophil and macrophage infiltration into mesenchyme; vasculitis with some thrombosis.	Hyperaemia of septal vessels with thrombus formation. Polymorph cell infiltration with necrosis; usually near stratum compactum. Bacteria commonly found in septal and blood vessels.

TABLE 8

Fetal lesions associated with abortion after experimental infection of pregnant ewes with L. monocytogenes

Macroscopic lesions	Microscopic lesions
Lung: Oedematous, with necrotic foci and haemorrhage. Autolysis present in some abortuses.	Broncho-pneumonia; oedema in inter-lobular septae. Scattered foci of organisms in colonies within airways, and blood vessels; neutrophil infiltration. Focal areas of necrosis surrounded by inflammatory cells.
Lung: Pale, necrotic foci, which may become generalised. Autolysis common, with congestion.	Foci of coagulative necrosis, proximal to blood vessels, containing bacteria. Usually lacks associated inflammatory changes. Macrophages in portal areas.
Kidney: Occasional pale foci of necrosis	Similar lesions to those found in the liver.

L.monocytogenes was inoculated via several different routes of administration into pregnant Suffolk ewes, and they aborted (Njoku and others 1972; Njoku and Dennis 1973a).

Variations occurred in this pathological picture according to the method of uterine inoculation. Using the intra-amniotic route of infection, caruncular lesions were minimal. After intra-fetal inoculation, cotyledonary autolysis was a common finding together with with hyperaemia or vasculitis; caruncular lesions were mostly those of hyperaemia with some bacteria present in septal walls.

Njoku and Dennis (1973b) have compared and recorded the histopathological lesions in aborted lambs following experimental and natural infections with L.monocytogenes. In

general, the lesions found associated with natural infections were similar to those recorded in cases of experimentally induced disease with intravenous inoculation of pregnant ewes. Any variations could be attributed to one of several factors, namely:

the susceptibility of the host.

the degree of bacterial challenge.

the route of infection.

time intervals between infection, onset of fetal disease and death.

Particularly significant were hepatic lesions related to field abortion cases, characterised by numerous focal necrotic lesions in various stages of development and adjacent to hepatic blood vessels. Broncho-pneumonia was more severe in fetuses aborted in the course of field infections, as were necrotic lesions in the placenta.

Osebold and others (1960) have given an account of the feto-placental lesions after experimental inoculation of pregnant heifers with L.monocytogenes and their subsequent abortion. Fetal autolysis was extensive, masking any gross ante-mortem lesions that could have been present: histologically, hepatic focal coagulative necrosis was obvious in association with clumps of bacteria. Within the placentomes there was a neutrophilic infiltration and some villous necrosis, with bacteria located at the materno-fetal interface.

Fungal infections.

The development of placental and fetal lesions associated with Aspergillus fumigatus infection and abortion has been determined from experimental infection of pregnant ewes (Cysewski and Pier 1968). Up to four days after infection, placental changes were observed in 25% of placentomes examined. Hyperaemia was predominant from the hilus to the base of caruncles. By the eighth day, 75% of placentomes were affected with zonary necrotic lesions; obvious thickening, oedema, and a red-brown exudate were found over the inter-cotyledonary areas. As the lesions aged, so the necrotic areas became irregular with a hyperaemic border. Up to 50% of the cut surfaces examined were affected.

The microscopic changes in the four-day lesion were characterised by a neutrophilic infiltration, congestion, and thrombosis of blood vessels in the distal half of the caruncular septa. Fungal hyphae were found in maternal blood vessels and septa, which then invaded the cotyledonary villi. Between eight and twenty-six days after infection, infarcts were located in placentomes where large areas of coagulative necrosis were boardered by zones of hyperaemia. Vasculitis and thrombus formation were common additional findings. Studies of the inter-cotyledonary chorio-allantois revealed areas of oedema, necrosis, and neutrophil infiltration with exudation; some blood vessels showed evidence of vasculitis with thrombosis. The chorionic

epithelium was lost at the maternal interface, and fungal hyphae were found in the surface exudate.

Gross fetal lesions were characteristic with this type of infection. Two types of skin lesion were found; the first consisted of raised, erythematous foci on the skin; the second were multiple yellow foci with erythematous borders which could coalesce. Multiple red focal lesions were usually found in fetal lungs.

On histological section, these skin lesions were described as an acute focal suppurative dermatitis with epidermal necrosis and thrombosis of the dermal vessels. Fungal hyphae were seen in hair follicles. In the lungs, an acute multifocal suppurative bronchitis was present with fungal hyphae in the air-ways; these hyphal elements extended into the surrounding parenchyma. Haemorrhagic infarcts with thrombosis affected inter-alveolar tissue; necrotic cells and neutrophil debris were prominent and fungal hyphae were also observed in lung capillaries.

Variations on this pattern of lesions have been recorded in field and experimental cases of fungal abortions in cattle. From his field investigations, Hillman (1969) described the gross cotyledonary lesions as being yellow to grey in colour with a marked thickening of the tissue, especially at the periphery. Hill and others (1971) described the same lesions as being white foci, but appearing as yellow necrotic strips on cut surface; these observations were made on bovine placentomes recovered after

experimental infection with A.fumigatus. Inter-cotyledonary lesions have consistently been described as thickened, wrinkled, leathery in appearance, with a yellow/grey or tan colouration (Pepin 1983; Canant 1985; Jerrett and McOrist 1985).

Canant (1985) described the gross appearance of the aborted fetus as being fresh with ringworm-like lesions on the skin surface. However, other workers have stated that only one-quarter to one-third of abortuses demonstrate visible skin lesions (Austwick 1968; Kirkbride 1975b; Werdin 1979). If they are present, the lesions are usually distributed cranio-dorsally (Dennis 1980a).

Regarding the microscopic findings, it has been shown that there is a good correlation between experimental and naturally occurring lesions (Hillman and McEntee 1969; Hill and others 1971; Jerrett and McOrist 1985). Miller and Quinn (1975) registered a marked folliculitis in fetal eyelid sections with a mixed inflammatory cell exudate which contained fungal hyphal elements in the keratinised epidermis, in 75% of fetuses that they examined. Other lesions have been described as in Table 9.

Mortierella wolfii has been reported as causing abortion in cattle in the United Kingdom (McDonald and Corbel 1981), but it seemed to be a more important pathogen for cattle in New Zealand (Menna and others 1972; Carter and others 1973) and Australia (Munday 1967; Skilbeck 1984), where the organism is regarded as the predominant cause of

TABLE 9

Occasional microscopic lesions associated with fungal abortions in cattle.

Morphological diagnosis	Histopathological findings	Reference
Necrotic meningoencephalitis	Coagulative necrosis with mononuclear and plasma cell infiltration in brain tissue. A mesenchymal cell proliferation of fibroblasts and astrocytes.	Guarda and others (1974)
Hepatic centri-lobular hyperplasia	—	Adamosteanu and Babi (1973)
Broncho-pneumonia	Suppurative bronchitis with exudate, neutrophils, and fungal hyphae in airways. Thrombus formation evident.	Dennis (1969) Kirkbride and others (1973) Adamkova and others (1981)

mycotic pneumonia in adult cows (Cordes and others 1972).

No gross fetal changes have been described, associated with M.wolfii infection in cattle (Neilan and others 1982) although Cordes and others (1972) have found focal lesions of hepatitis after experimental infection in pregnant cattle. The changes could not be detected macroscopically in the acute stages of infection, but well-demarcated pale-yellow foci were seen as chronic lesions. Microscopically, the lesions consisted of focal areas of coagulative necrosis surrounded by clumps of neutrophils and fungal elements.

In both experimental and field infections, microscopic lesions of fetal encephalitis have been detailed (Cordes and others 1972; Neilan and others 1982), characterised by vasculitis, malacia, and haemorrhage with an abundant neutrophil infiltration and hyphal invasion. Cerebral and meningeal blood vessels were often thrombosed, and the capillary walls invaded with fungal hyphae.

The same authors have also recognised gross lesions in the placenta in these same cases. The placentitis was characterised by a thickened, patchy, red/yellow discolouration of some caruncles, and wedge-shaped infarcts were present. Histologically, the caruncular lesions were essentially a necrosis of the stromal tissue with thrombosis which was located near the arcuate arteries of the hilus and along the trabeculae.

Organisms of the genus Mycoplasma.

The Mycoplasmas associated with abortion in cattle have been identified as M.bovis, Acholeplasma laidlawii (Langford 1975; Ball and others 1978; Bocklish and others 1981; Stipkovits and others 1981), M.bovigenitalium (Hirth and others 1970), M.agalactia subsp.bovis (Stalheim and Procter 1976), M.alkalescens (Rosenfeld and Hill 1980), and Ureaplasma diversum (Ruhnke and others 1984). In a study of 245 bovine abortions, Ball and others (1978) recovered Mycoplasmas from 23.7% of fetuses examined.

Miller and others (1983c) have published the results of

experimental inoculation of U.diversum into the amniotic cavity of four pregnant cattle where the outcome was either abortion or stillbirth. A diffuse thickening of the amnion with some fibrosis or mineralisation was recognisable, the microscopic lesions being a chronic active amnionitis characterised by fibrosis, mineralisation, a heavy mononuclear cell infiltration, and foci of haemorrhage and necrosis. There were also microscopic changes in the chorio-allantois, a mild vasculitis and heavy mixed inflammatory cell infiltration within necrotic villi.

The only lesions identified in the fetus were microscopic, and they were confined to the lung. They varied from mild to severe lymphocytic peri-bronchiolar cuffing with a diffuse alveolitis. Alveolar septa were thickened by mono-nuclear cell infiltration. Ruhnke and others (1984) have subsequently reported mild accumulations of mononuclear cells in the hepatic portal areas of fetal livers from two abortuses, and one observation of a focal lymphocytic myocarditis.

Miller (1984) has identified specific lesions in eyelid of fetuses that were experimentally infected with U.diversum. The characteristic feature of this lesion was multiple goblet cell formation with pseudo-stratification of the conjunctival epithelium and sub-conjunctival mononuclear cell infiltration.

Bocklish and others (1981) have given details of pathological changes in fetuses that have been aborted in

outbreaks of Mycoplasma infections in cattle; comment on placental pathology was not included. Fetal lesions were restricted to the lungs, liver, and reticulo-endothelial system. Characteristic lung changes were of oedema in the inter-lobular septa with some hyperaemia, and inflammatory cell exudate was found in airways. The epithelium which lined the bronchi had undergone desquamation. In the liver there was thrombus formation of the centri-lobular vein, which was surrounded by lymphocytic and plasma cell infiltrations. The trabeculae within the parenchyma were congested, and oedema with an inflammatory cell infiltration was located in the peri-acinar region of hepatic lobules.

Rosenfeld and Hill (1980) found similar lung lesions associated with M.alkalescens infection and abortion, as well as thymic medullary enlargement and loss of differentiation between cortex and medulla.

Bacteria of the genus Pasteurella.

Bacteria of this genera are associated with the clinical conditions of Haemorrhagic Septicaemia and Transit Fever (or Shipping Fever) in cattle, the aetiological agent in this instance being Pasteurella multocida. The same organism is also a secondary bacterial invader in cases of primary viral pneumonias of young cattle (Dungworth 1985). P.haemolytica infections have been closely linked to fatal septicaemias of feeder lambs. Only recently has P.haemolytica been isolated from two aborted bovine fetuses

in Northern Ireland (Neill 1981). From North America come reports of Pasteurella organisms being connected with several field outbreaks of abortion (Ward and others 1985), which appeared to be sporadic in incidence, and capable of occurring during any stage of pregnancy. However, high maternal blood titres to H.somnis and B.V.D virus were recorded concurrently in most of the cases so far studied.

The histological lesions presented by the fetuses under investigation were a diffuse oedema and mild congestion in lungs, with an acute pneumonitis. There were also increased numbers of macrophages, neutrophils, and fibrin deposits in the alveoli. No other lesions were described.

Bacteria of the genus Proteus.

Members of the genus Proteus are ubiquitous and are so frequently found as contaminants in bacterial cultures of abortion material that they are seldom considered to be of aetiological importance in outbreaks of bovine abortion. However, Sheleu and others (1969) isolated Proteus vulgaris from six bovine fetuses after spontaneous abortions. The fetal lesions that they described included a hyperaemia in liver sinusoids with diffuse fatty change in hepatocytes, and hyperaemia of lung and heart tissues. Placental lesions were non-specific, such as oedema and hyperaemia. Necrosis of placental epithelial cells, thrombus formation, and occasional bacterial emboli were seen in blood vessels of the intima.

Bacteria of the genus Salmonella.

Bovine Salmonellosis is a disease encountered world-wide. Abortion is but one manifestation of the clinical syndrome displayed in affected herds and, in many instances, is confined to sporadic incidents. Hinton (1971) has reviewed the serotypes associated with abortion episodes and found that Salmonella dublin was the predominant isolate, but many other serotypes have been cultured directly from abortion material. A diagnosis of Salmonellosis has usually been based upon the isolation of bacteria from an aborted fetus or its placenta, but mixed infections do occur.

Frik (1969) described the epidemiology of the infection in Holland and showed that not only was the disease localised to geographical areas of the country, but that the highest incidence occurred in the latter months of the calendar year. Field (1949) has given a classical account of the clinical manifestations of the condition, together with a commentary on the incidence of abortions when related to the presence of concurrent enteric symptoms within affected herds.

Experimental infection of pregnant cattle with S.dublin has been shown to cause pyrexia followed by abortion (Hall and Jones 1976), but no pathological features were described other than a high incidence of retained fetal membranes.

In his review, Hinton(1971) stated that there did not appear to be any specific pathological lesions associated

with Salmonella abortions, either in the fetus or placenta. Generalised gross features presented by aborted fetuses could be subcutaneous oedema and accumulation of sero-sanguinous fluid in the peritoneum; placental changes were oedema and a yellow/grey appearance, streaked with pus-like material (Deas 1984). Russell (1973) found placental lesions in one out of three abortions which occurred in a 140-cow dairy herd in Victoria, Australia, associated with S.dublin infection. Gross findings were a necrotic placentitis in cotyledonary tissues, but microscopic necrosis involved only the chorionic epithelium, there being minimal inflammation of the underlying tissues. Bacterial colonisation of cotyledonary villi was evident, accompanied by a mild polymorphonuclear inflammatory cell infiltration. Any heavy mixed inflammatory cell infiltrations that were seen were near to blood capillaries, whilst some larger vessels showed thrombosis and inflammatory changes in their walls.

Protozoa of the genus Sarcocystis.

Certain species of this organism have been found in skeletal muscle of cattle but were considered to be non-pathogenic. The primary hosts of these protozoans are dogs, cats, and primates (Stalheim and others 1980). Experimental infection of pregnant cattle with Sarcocystis cruzi has induced abortion (Barnett and others 1973), but no fetal lesions were described. Recognisable lesions of an endometritis and placentitis were found which involved

numerous protozoan cysts.

Dubey and Bergeron (1982) reported an abortion induced by Sarcocystis in one pregnant cow, and described the development of the parasite within the fetal tissues and placenta. Other single cases have been reported by Hong and others (1982) and Vickers and Brooks (1983). No gross lesions have been descried, except for those found in fetal liver by Hong and others (1982) where the liver was said to be granular in appearance with a thin white outline to its lobular structure; other fetal organs were autolysed.

The histological lesions observed by these two groups of workers are summarised in Table 10.

Similar lesions were observed in fetal lungs by Vickers and Brooks(1983), but with no accompanying inflammatory reaction.

TABLE 10

Histological lesions recorded in aborted fetal tissues associated with sarcocystis infection of cattle.

Tissues	Dubey and Bergeron (1982)	Hong and others (1982)
Brain	—	Microgranulomas and glial nodules with central necrosis and peripheral aggregates of glial cells; granulomas encompassing capillaries contain free merozoites. Perivascular cuffing with mononuclear cells. Capillaries occluded by schizonts.
Liver	Autolysed	Schizonts in vascular endothelium; mononuclear cell infiltration around hepatic portal tracts.
Kidney	Focal necrosis and mononuclear cell infiltration in glomeruli and inter-tubular connective tissue of cortex and and medulla. Protozoa in endothelial cells of glomeruli and arterioles in medulla.	Similar to the liver, but mononuclear cells clustered around blood vessels in the interstitium.
Lung	Congestion and oedema. Focal vasculitis. Protozoa in endothelial cells of capillaries and arterioles.	Schizonts in vascular endothelium.
Heart	—	As for the liver, but mononuclear infiltration associated with blood vasculature.
Placenta	Multiple focal necrosis, involving lamina propria, with mixed inflammatory cell infiltration. Protozoa seen in lamina propria of villi and small arterioles.	—

Akabane virus.

This virus is a member of the Simbo group of the Bunyaviridae. It has been said to cause fetal intra-uterine infections in all the domesticated ruminants which can result in abortion, premature births, stillbirths, and fetal deformities such as congenital arthrogryposis-hydrancephaly syndrome. The disease has been described as being of seasonal epidemic proportions in Japan (Ohashi 1973), and has also been reported in Israel (Nobel and others 1971) and Australia (Hartley and Wanner 1974).

Experimental intravenous infection of pregnant ewes with the B8935 strain of virus produced a variety of fetal lesions, depending on the stage of gestation at which the the fetus was exposed to infection. In two dead fetuses, scoliosis, kyphosis, brachygnathia, and hydrancephaly were gross features. Some lambs were born alive, but with small heads and mild inco-ordination; micrencephaly and porencephaly were found at autopsy (Parsonson and others 1975).

The only microscopic inflammatory lesions that have been reported were found in the central nervous system; they include perivascular cuffing, neuronophagia, and glial cell proliferation, and were only found in the early stages of an epidemic (Nosaka and others 1973).

Bovine Para-influenza 3 (PI3) virus.

PI3 virus infection in cattle is widespread, and causes a mild inflammatory condition of the upper respiratory tract

in young-stock. There is a low mortality rate with uncomplicated infections (Woods 1968).This virus has been recovered from field outbreaks of abortion in cattle (Satter and others 1965), and has been shown to cause abortion following experimental fetal inoculation of virus around mid-gestation (Swift and Kennedy 1972).

Swift and Trueblood (1973) have given an account of the gross lesions found in two fetuses born at term, after being experimentally infected during pregnancy. These calves were weakly and non-viable, with clinical signs of bilateral keratitis, anterior uveitis, and congenital cataracts. Pneumonic lesions were present post mortem.

Microscopic lesions that were found in infected fetuses were of a lympho-reticular hyperplasia throughout the Reticulo-endothelial System. Aborted fetuses showed distinctive lesions of a necrotising bronchiolitis and patchy interstitial pneumonia. Peri-bronchiolar lympho-reticular proliferation was located in lungs twenty days after inoculation (Swift 1973).

Bovine Parvovirus.

Parvovirus infection of the large domestic animals has been most commonly associated with reproductive disease in pigs (Johnson and Collings 1969) but rarely with sporadic field cases of abortion in cattle (Inaba and others 1973).

Storz and Young (1980) have reported abortions in cattle following direct fetal inoculation with parvovirus

strain BPV-4 during the first and second trimesters of pregnancy. They described the susceptibility and immune response of bovine fetuses to this infection as being similar to that reported for fetal infection with Bovine Viral Diarrhoea virus infection. Pathological lesions were found in aborted fetuses from the second trimester of pregnancy. They included intra-nuclear inclusion bodies within multi-focal areas of adrenocortical necrosis, as well as focal necrosis and depopulation of the external sub-pial germinative granulosa cell layer of the cerebellar cortex.

Liggitt and others (1982) inoculated 130-day fetuses with parvovirus and found no gross lesions. Their major findings were microscopic lesions of extra-medullary haematopoiesis in all lymph nodes and spleen, where well-defined accumulations of peri-arteriolar lymphocytes were placed. These changes were regarded as non-specific, because they accompanied the development of neutralising antibodies in the fetuses.

Bovine Viral Diarrhoea (BVD) virus.

BVD virus is an enveloped R.N.A virus of the family Togaviridae, genus Pestivirus (Porterfield and others 1978). Viral isolates are loosely classed as either cytopathic or non-cytopathic, depending on their cytopathogenicity in cell culture (Heuschele 1975).

The virus was first recognised as exerting an adverse effect on cattle productivity by Olafson and others (1946).

Since then, it has been linked to a variety of syndromes, described as follows:

i) an acute, fatal disease with fever, profuse diarrhoea and buccal ulcers.

ii) a chronic, wasting disease with persistent diarrhoea. Ulceration in the mouth and inter-digital areas is common.

iii) respiratory disease.

iv) fetal mummification, abortion, stillbirths, congenital malformations, and birth of underweight-for-age fetuses. (Ruth 1986).

The virus has an affinity for, and a direct necrotising effect upon, a variety of tissues:

i) squamous epithelium of coronary bands, inter-digital skin, and upper respiratory tract.

ii) epithelium lining the lower alimentory tract, respiratory tract, and renal convoluted tubules.

iii) endothelial cells of blood vessels in small organs, especially the intestine.

iv) lymphoid tissue, especially tonsils and Peyer's patches.

v) neurons.

The tissues of the fetus are at risk from the virus because of trans-placental spread of infection (Barker and van Dreumel 1985).

The effect of the virus has the fetus depends on its gestational age. Abortions have been reported in non-immune,

pregnant cattle infected from days 50-100 of pregnancy (Kahrs 1981). As the fetus gains immunological competence to respond to different antigens in a step-wise fashion, it has been suggested that the most common result of congenital B.V.D viral infection is sub-clinical, non-fatal disease (Schultz 1973). Should the fetus die, the end result is usually abortion (Kendrick 1984).

Fetuses which have been infected during the second trimester of pregnancy often survive but may develop congenital defects. Because the virus damages the rapidly dividing cells of various organs, such teratological abnormalities differ according to the time of fetal infection. A number of gross pathological entities have been associated with B.V.D virus infection in both cattle and sheep fetuses:

mandibular brachygnathism	Scott; Terlecki.
cerebellar hypoplasia	Scott; Terlecki.
mummified fetus	Scott; Terlecki.
persistent pupillary membrane	Scott; Brown.
retinal atrophy/cataract	Scott; Brown.
small optic nerve	Scott; Brown.
reduction in long-bone size	Terlecki.
narrow renal cortex	Terlecki.
skin abnormalities	Ohmann.
sero-fibrinous arthritis	Ohmann.
lymph node/thymic hyperplasia	Ohmann.

(references: Scott and others (1973); Terlecki and others (1980); Brown and others (1974); Ohmann (1982).

There are no accounts given of fetal microscopic lesions acquired during early pregnancy. Those that are found later on in pregnancy are usually mild enough for the fetus to survive, and are limited to the blood vascular system. Placental vasculitis is a common lesion characterised by a mononuclear cell infiltration, as is reticulo-endothelial hyperplasia in the portal areas of the liver, and in lymph nodes. The thymus may be hypoplastic. After experimental infection of pregnant cattle, the caruncles recovered during Caesarian section at the birth of these infected calves showed evidence of a mild non-suppurative arteritis (Kendrick 1984).

TABLE 11

Histological lesions associated with experimental BVD virus infection of bovine fetuses.

Tissue	Lesion	Description
Cerebellum	Cerebellar hypoplasia	Necrosis of cells in external germinal layer Infiltration of mononuclear cells in Leptomeninges Degeneration and depletion of Purkinje cells.
Hard palate buccal mucosa	Epithelial degeneration	Vacuolar/hydropic degeneration
Lung	Increased interstitial cellularity	Marked peri-bronchiolar lymphonodular hyperplasia
Lymph nodes	Precocious development	Cortico-medullary zone characterised by dense population of small lymphocytes. Zonary expansion of non-lymphoid cells. Marked lymphoblastoid reaction.
Skin	Dermal necrosis	Epithelial vacuolar/hydropic degeneration, with hyper- and para-keratotic lesions. Subcutaneous infiltration by mononuclear and plasma cells

Kendrick (1971) has described skin lesions in a calf, born with only a thin distribution of hair over its body, and that of irregular length. Biopsy showed a follicular hypoplasia and cystic dilation of the adnexal glands.

Other histological lesions have been described, after similar experimental methods to those recorded above, by Ohmann (1982) and are summarised in Table 11.

Cytomegalovirus.

Infections with Cytomegalovirus are common in man, producing four forms of inclusion-body disease:

i) infection of adults with absence of symptoms; pathological changes occur only in the salivary glands.

ii) infection of children and adults, involving intestine and/or lungs; other debilitating diseases may also be present.

iii) infection in infancy or early childhood involving lung or intestine; death occurs from either viral infection itself or associated disease.

iv) infection of the fetus; usually fatal, or congenital malformations common-place. Plummer (1973).

Pathological findings associated with cytomegalovirus infection calf have been recorded by Schiefer (1974), in one aborted calf at 28 weeks gestation, born in a herd of 50 Hereford cows in British Columbia. A diffuse proliferation of alveolar cells was seen in fetal lungs together with

numerous large cells, containing prominent intranuclear inclusions. Similar inclusion bodies were also located in bile duct epithelium, myocardium, and spleen. Within affected kidney tubules, enlarged cells which also contained inclusion bodies appeared to develop in groups. These changes were similar to those found in infant fetuses suffering from this infection (Potter 1961).

Herpes virus.

Herpesvirus bovis is the aetiological agent of the disease Infectious Bovine Rhino-tracheitis (I.B.R), a condition characterised by clinical signs of acute upper respiratory tract infection, accompanied by a severe conjunctivitis and/or a pustular vulvo-vaginitis (Miller 1955). This virus has caused abortion in non-immune pregnant cattle (Chow and others 1964).

It would appear that placental infection with Herpesvirus is unique, in that the virus does not pass immediately to the fetus after maternal infection. Initially, trophoblast cells appear to contain the virus, but it then spreads slowly from cell to cell (Kendrick 1973). Hence in naturally occurring infections, the period of time between initial exposure of the pregnant dam to Herpesvirus and subsequent abortion is 18-90 days. Abortions in cattle are usually confined to animals pregnant over 165 days.

When the placenta does release the virus into the feto-placental circulation a peracute disease precedes fetal

death by 24-48 hours, with widespread tissue necrosis (Kendrick and Straub 1967). If the fetus is challenged nearer to full term, its susceptibility to the virus becomes more like that of the neonate.

Fetuses aborted as a consequence of Herpesvirus infection are invariably autolysed, with an accumulation of

TABLE 12

Some fetal and placental lesions associated with Herpesvirus infection of pregnant cattle and subsequent abortion

Tissue	Description of lesion	Authors/reference
Palpebral	Epithelial degeneration / necrosis	5
Conjunctiva	Mononuclear cell infiltration	5
Lung	Alveolar oedema	4
	Meconium/squames in airways	4
	Mineralisation and focal necrosis	2 4 5
	Alveolar mononuclear cell infiltration	4
	Mixed inflammatory cell exudate	5
Heart	Focal degeneration/necrosis of myocardium	4
Liver	Coagulative necrosis	1 2 3 4 5
	Periportal mononuclear cell infiltration	2 3
Kidney	Focal haemorrhagic necrosis in renal cortex medulla	1 2 3 4 5
Adrenal	Multifocal necrosis in cortex	3 4 5
Spleen	Coagulative necrosis	2 5
	Lymphocytic depletion	2
Placenta	Neutrophil accumulation with focal/diffuse necrosis	1 4
	Vasculitis and mineralisation	4

Key to numbers for references:

1 Kennendy and Richards (1964)
2 Owen and others (1968)
3 Kendrick (1973)
4 Miller and Quinn (1975)
5 Miller and others (1978)

dark-red fluid in subcutaneous tissue and body cavities. This may indicate that death occurred 5-7 days prior to the abortion (Kendrick 1980).

Pathological lesions that have been described in cases of fetal and placental Herpesvirus infection are summarised in Table 12.

MATERIALS AND METHODS.

3.1. Source of material.

The abortion cases which make up this study were taken from herds belonging to clients of a six-man agricultural practice in the Lune Valley, situated on the Lancashire/ Cumbrian border.

The majority of farms were medium-sized dairy units with an average herd size of 60-70 milkers.

3.2. Acquisition of cases.

A news-letter was circulated monthly to all farmer clients of the practice. A paragraph in the June 1984 edition encouraged farmers to report all incidents of bovine abortion to the practice.

The investigative procedure was designed so as not to impose any additional restrictions, other than those already in force under the Brucellosis (England and Wales) Order 1978.

Cases described in the study all originated from clients voluntarily reporting abortion incidents on their farms to the veterinary practice.

3.3. On-farm investigative procedure.

The procedure was designed to fulfil the requirements of the Brucellosis Order, and every incident was investigated through completion of Form BS7 (see Appendix 1). The required samples of a maternal blood sample, milk, and

vaginal swab from the aborting cow were sent to the local Veterinary Investigation Centre at Penrith, either by post or bus. In order to obtain additional information as to a possible aetiology for the abortion incidents investigated, fetus, placenta, and an additional maternal blood sample were collected. The complete schedule is summarised in Table 13.

TABLE 13

Summary of sampling procedure for investigation of abortion in dairy cattle.

sample obtained	Mandatory (M) or Additional (A)	Method of collection	Destination of sample
Serum (7 mls)	M	Vacutainer (Red top)	V.I.C.
Milk	M	Plastic bottle	V.I.C.
Vaginal swab	M	Sterile swab	V.I.C.
Fetus (where available)	A	Plastic sack	Practice lab.
Placenta	A	Plastic bag	Practice lab.
Caruncle (where available)	A	Disposable glove	Practice lab.
Serum (7 mls)	A	Vacutainer (Red top)	V.I.C.
Serum (7 mls), four weeks after initial visit	A	Vacutainer (Red top)	V.I.C.

All samples were routinely identified from the ear-tag number of the aborting cow, the farm of origin, and the date. They were taken to the practice laboratory within two hours of collection.

Clients were provided with a written report of the laboratory findings and any conclusions which could be reached on completion of the investigations.

3.4.**Practice laboratory procedures**.

The methods used were based upon a systematic, multi-level diagnostic procedure outlined by Kradel (1978) and described by Kirkbride (1984a,b,c).

i) Blood samples.

7ml of whole clotted blood was allowed to stand for six hours at 2-8 C, so as to form a firm, contracted clot. It was then centrifuged at 2500g for four minutes and the serum was drawn off using a glass pipette. Serum was placed in 5ml plastic containers without anti-coagulant (Monoject Z/5.BS 4851). It was stored in a deep freeze until two blood samples had been collected from each case, processed, and then submitted to the V.I. Centre.

ii) Post mortem examination of fetuses.

A measure was made of the crown-rump length of each fetus in centimetres using a steel tape-measure, after the method of Evans and Sack (1973). The carcass was weighed in kilograms.

Any gross external abnormalities were recorded on a post mortem form (Appendix 2).

Note was made of the presence or absence of meconium staining and areas of discolouration or matting in the hair.

Carcases were placed in dorsal recumbency and a routine post mortem technique was performed, similar to that described by Doxey (1983). The abdomen was

opened by a mid-line incision and skin and abdominal muscles were reflected, assisted by an incision just caudal and parallel to the last ribs. The abdominal contents were displayed. The mid-line skin incision was extended cranially to the level of the angle of the mandibles. The skin, fascia, and muscles were reflected back to expose the sternum, chest wall, and deep structures of the neck. The sternum was removed at the costo-chondral junctions, together with its covering of fascia and muscle. The contents of the thorax were exposed, together with the ventral cervical portion of the thymus gland. A ventral incision over the trachea and oesophagus was extended upwards to the larynx, and the thyroid gland was exposed, lying lateral to the thyroid cartilages of the larynx. The contents of abdomen and thorax were examined in situ for any gross abnormalities. Routine samples were then collected from:

a) abomasum: 7ml of contents were collected in an evacuated glass tube (Vacutainer).

b) thorax: approximately 2-7ml of fluid was collected in a Vacutainer before the lungs or heart were removed for examination.

The abdominal viscera were then removed and examined in detail. Descriptions were made of all tissues. Routine samples were taken from:

c) liver: approximately 200 gm of fresh liver was

placed in a polythene bag and refrigerated under ice.

d) kidney: approximately 70gm of fresh tissue was treated in a similar fashion to liver.

Bacteriological samples were now obtained from abomasal contents, liver, lung, and cotyledon or maternal caruncle if available, as described by Randall (1954) and Holter and Andrews (1979). Single agar plates were sub-divided to allow up to three tissue samples to be screened from one abortus at the same time. Drops of fluid or portions of tissue were placed on Blood Agar and McConkey's media, and a sterile loop was used to draw out the inoculum which remained, after the method of Konemann and others (1983). McConkey plates were incubated aerobically at 35 C for 48hrs in a laboratory incubator: blood agar plates were similarly incubated under partial anaerobic conditions of 5-10% carbon dioxide. All plates were read by a laboratory technician at the end of the incubation period. Bacteria taken from representative colonies of those grown were stained by Gram's method for identification. All unidentified bacterial colonies were sub-cultured onto the original medium and submitted to the V.I.Centre. Penrith for identification.

The contents of the thorax were then examined in detail, particular attention being paid to the heart and great vessels.

The interpretation of the bacteriological findings was based on the method outlined by Miller and Quinn (1975); an agar plate containing over 100 colonies of one bacterial type was classified as a pure culture, whereas a plate containing two or more types of colony was designated a mixed culture. The present study used a critical count of 30 colonies of one type, because single agar plates were sub-divided into three parts.

Portions of tissue were removed for histological examination, after the method of Randall (1954). Small blocks of selected tissues were placed in 10% formalin, the routine samples being from liver, heart, lung, eyelid, caruncle, and cotyledon. Additional portions of tissue were taken from organs which showed evidence of gross pathological change. Finally, the post mortem examination form (Appendix 2) was completed for each case, which also served as a check to ensure that the procedure had been completed.

3.5. **Submission of samples to Veterinary Investigation Centres.**

a) for Brucellosis testing.

These mandatory samples were packaged in boxes supplied by M.A.F.F. and sent by post to the particular Veterinary Investigation Centre which

serviced the administrative area in which the farm was located, where the abortion had occurred.

Farms in:-

i) North Yorkshire were serviced by Leeds V.I.C.

ii) Lancashire were serviced by Liverpool V.I.C., Crown Street, lately moved to Barton Hall, Preston.

iii) Cumbria were serviced by Penrith V.I.C.

b) additional pathological material.

The fetal tissues collected at post mortem, namely thoracic fluid, abomasal contents, fresh chilled liver and kidney, together with the formalinised tissue blocks, were packaged in leak-proof boxes suitable for carriage by bus to Penrith V.I.C.. The journey time was three hours. A completed form VIO 16 (Appendix 3) accompanied each case submission, which also included data on the gross post-mortem findings. Bacterial cultures on blood agar or McConkey plates which required identification by V.I.C. staff were similarly transported.

3.6. **Veterinary Investigation Centre laboratory procedures.**

All material received was labelled and identified with the relevant abortion case number (F....) or maternal serum number (C...), super-imposed on submitted form VIO 16. The reporting of the laboratory findings was made back to the practice using this combined method of identification, using

a standard format for presenting results (see Appendix 4-6).

i) fetal serology: fetal thoracic fluid was examined for evidence of Infectious Bovine Rhino-tracheitis Virus and Bovine Viral Diarrhoea Virus using a Serum Neutralisation Test, for Leptospira hardjo using a Microscopic Agglutination Test, and for the presence of IgG and IgM immunoglobulins by a single radial immunodiffusion test (R.I.T) using commercially available test kits (Miles Laboratories, Slough, England.)

ii) Fluorescent Antibody Test (F.A.T): fresh, chilled fetal liver and kidney were minced to prepare tissue homogenates. Leptospira F.A.Ts were carried out on both tissue homogenates, as described by Ellis and others (1982).

iii) maternal serology: the following tests were carried out on paired maternal sera:-
M.A.T. for Leptospira organisms (Wolff 1954).
Either a) S.N.T. or b) ELISA plate test using a conjugate of cow immuno-ferroxidase conjugated rabbit immunoglobulin (Dakopath p159) and O-phenyl-enediamine substrate, for I.B.R and B.V.D.

iv) preparation of histological sections: the tissues were trimmed and embedded in paraffin wax as multiple tissue blocks. They were sectioned at 6-8u thickness and stained with haematoxylin and eosin. After mounting, the relevant Case Number was etched

onto each glass slide.

Prepared slides were examined by the author using a Zeiss laboratory microscope, with x10 eye-piece and x6.3, x10, and x40 objective lenses. A full description of the histological sections was recorded for each case.

All case findings were then assessed, taking into account the V.I.Centre and practice laboratory findings, together with the pathological observations. The results of the completed investigation were sent to the client concerned.

RESULTS.

Of 149 abortion episodes investigated on 54 farms with an estimated breeding cattle population of some 3665 animals, 122 fetuses were born dead. 11 fetuses were born alive but only five survived. Those calves which died within 48 hrs of birth were examined post-mortem as described. Six sets of twins were aborted. In eight additional cases a mummified fetus was delivered and only a cursory examination was made. In 24 investigations no fetus was recovered; in nine of these cases, pathological examination of placental membranes or caruncle was the only possibility.

4.1. Gross pathological findings in aborted fetuses.

a) Crown-rump length.

The crown-rump length of 136 aborted fetuses was recorded in centimetres, as an indication of the gestational ages of the fetuses examined. The actual values that were obtained are plotted in Figure 4-1, alongside a computer-based curve showing the relationship between crown-rump length and fetal age for Friesian cattle (Richardson and others 1982).

b) Fetal weights.

The weights of 122 dead fetuses were recorded in kilograms on weigh-scales in the laboratory. The relationship between gestational age and birth weight is described in Figure 4-2 alongside a computer-based curve of the same data showing growth rates of healthy Friesian fetuses (Richardson and others 1982).

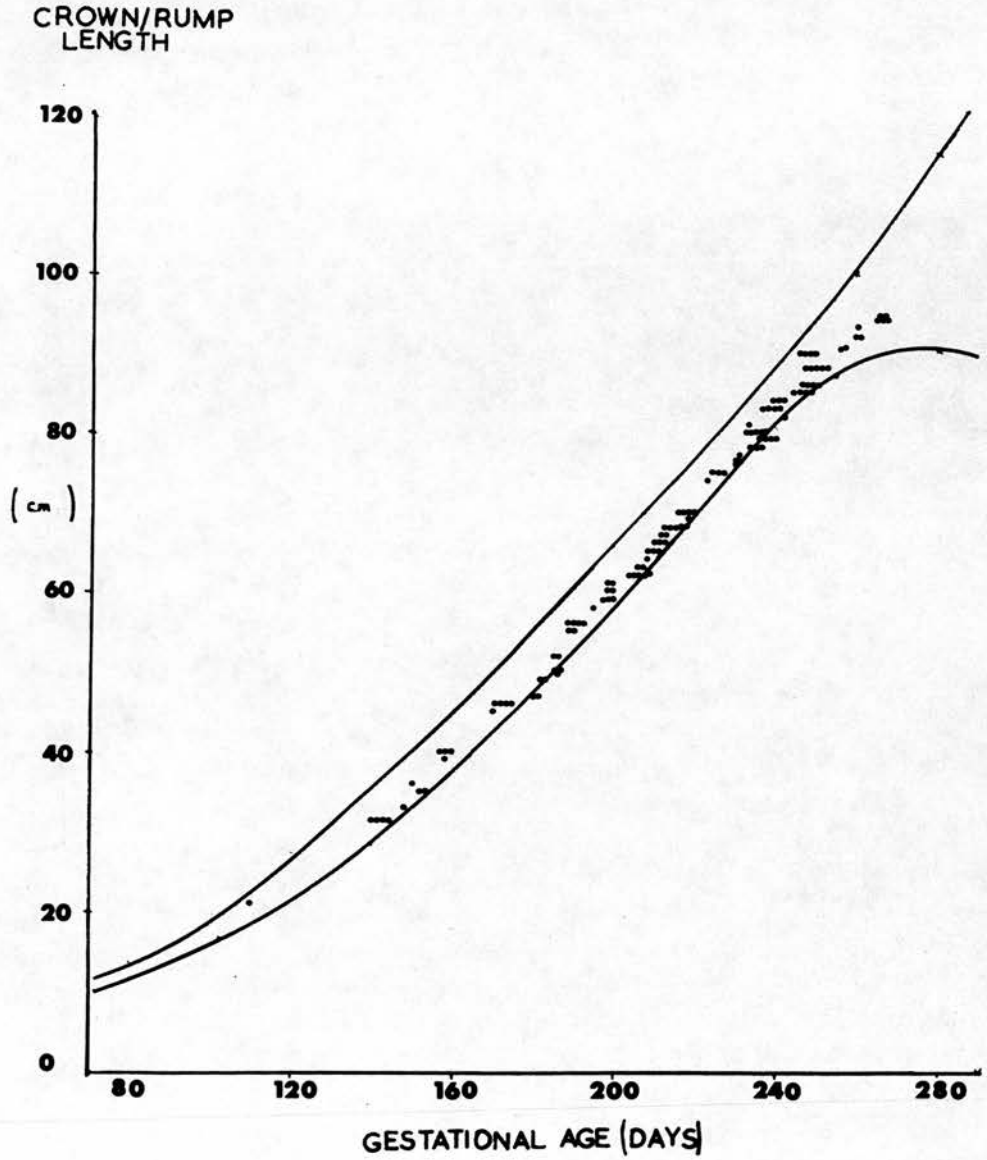

Fig. 4-1 Gestational age (days) of 136 aborted fetuses, estimated from crown-rump length (cm) measured at birth.

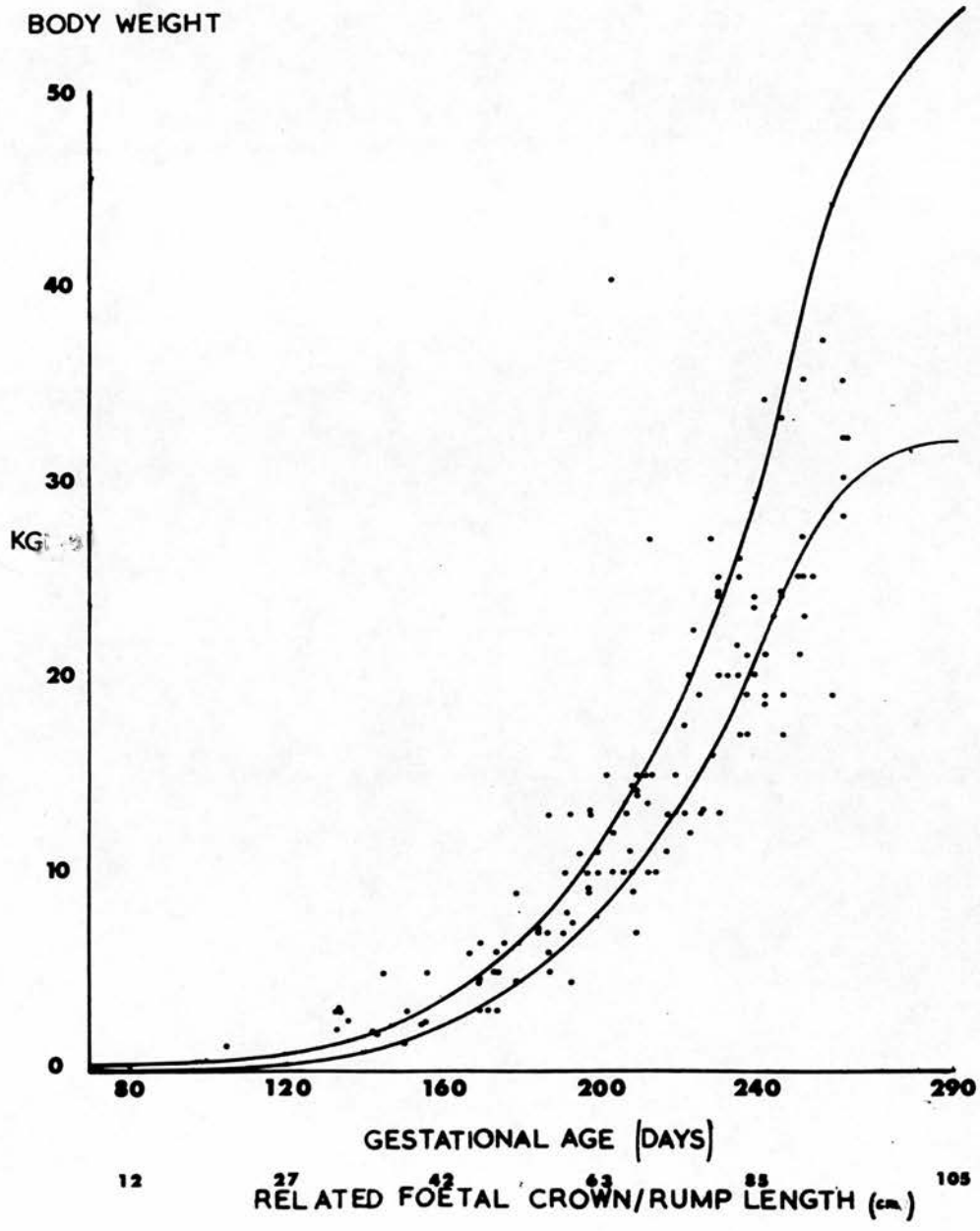

Fig. 4-2 Birth weights of 122 aborted fetuses (kg) in relation to their gestational age as estimated by crown-rump length (cm).

Eleven fetuses were recorded as being under-weight for age and possible causes of these abortions are recorded below:

Bovine Viral Diarrhoea Virus (B.V.D).	4 cases.
Bacterial septicaemia	3 cases.
Leptospira hardjo.	1 case.
No diagnosis	3 cases.

c) Autolysis.

Of 122 fetuses examined, 81 presented some evidence of autolytic change. 36/81 carcases displayed generalised autolysis as characterised by subcutaneous tissue discolouration and reddish fluid accumulation, some tissue pallor of liver and heart, or the variable finding of gas bubbles in subcutaneous tissues, liver, kidney, and skeletal muscle.

Autolytic changes in 45/81 of fetuses were confined mainly to the kidneys, where tissue softening or partial liquefaction were most commonly found. Of these cases, 29 presented additional post-mortem changes in the liver, with some tissue softening, pallor, and/or yellow-brown staining from bile seepage.

d) Fluid in body cavities.

Red-tinged fluid had collected in both abdominal and thoracic cavities of 111 fetuses examined. In carcases undergoing generalised autolysis the volumes of fluid were large, being up to 200ml in thorax or 5 litres in abdomen.

It was sero-sanguinous, dark-red in colour, indicative of intra-vascular haemolysis and leakage. In the remainder, fluid accumulations were reduced by comparison; amounts averaged 150mls in the abdomen and 20mls in the thorax. Fluid found in the thorax was usually less heavily stained than that in the abdomen, being a pink to rose colour. In one exceptional case (C130), over 17 litres of clear-to-pink fluid was found in both body cavities and associated with fetal anasarca; in another (F1796), 15 litres of similar material was collected from the abdomen of a fetus suffering from ascites.

e) Oedema.

Generalised oedema was found in three abortuses. An excess of clear or straw-coloured fluid was located within subcutaneous tissues as well as abdominal and thoracic cavities. Inter-lobular oedema of the lungs was also a prominent feature.

Localised oedema of the head and neck was observed in five fetuses.

In 24 pairs of lungs examined, inter-lobular oedema was a feature which was apparently associated with concurrent localised autolysis of the fetus.

Oedema was also present in fetal membranes from 18 abortions which were examined, and within the caruncles of a further 3. In two carcases (F1336:F1462), hepatomegaly was a feature which caught the eye, without autolysis being found except for early changes in the kidneys. These findings,

in Table 14. The raw data which provided these results, and case inter-relationships within the tables and figures may be found in Appendix 8.

TABLE 14

Relationship of oedema in fetal and maternal placental tissues, and gross pathological changes in aborted fetuses

Total	Oedema observed in :	Autolytic change in Liver & kidney	Autolytic change in Kidney	Hepato-megaly
12	Inter-cotyledonary chorio-allantois	4	4	6
6	Amnion	3	1	2
3	Caruncle	-	-	2

f) External fetal abnormalities.

The gross anatomical appearance of 15 fetuses was considered to be abnormal, as summarised in Table 15.

TABLE 15:

Abnormal external features of 15 aborted fetuses found at post mortem examination

Abnormality	Number of fetuses
Meconium staining of hair	6
White plaques on skin/hair	2
Ascites	1
Anasarca	2*
Congenital cataracts	1
Skeletal deformities:	
over-shot jaw	2
shortening of muzzle/ doming of skull	2*

* indicates one fetus with a common abnormality.

Meconium staining was frequently found on the inner aspects of thighs of fetuses, extending towards the hocks. In two cases there was extensive staining of the coat-hairs covering the whole body.

White plaques were found on the hair or skin of three fetuses (F1981;F2879;F3023), located in discrete areas of the shoulders and chest, and matted into the skin hairs that were present.

Congenital cataracts that were found in one fetus (F1065) coexisted with an abnormality of the spiral colon. The intestine terminated in a blind-ended diverticulum with gut proximal to the lesion dilated by an accumulation of digesta. The distal part of the colon and rectum was empty save for a clear, gelatinous, mucoid secretion within the lumen.

g) Pathological findings in fetal abdomen and its contents.

Gross pathological features that were recorded in 96 of the abortuses examined are set out in Table 16. Two cases of hepatomegaly are included within the group of cases described as showing gross liver enlargement, the weights of the livers being 2.4kg (F887) and 3.4kg (F309) in fetuses that weighed 10kg and 14kg, respectively. In one additional abortion (C130), a total fetotomy was necessary in order to deliver a fetus with anasarca where the carcase weight was estimated at over 40kg and the liver weighed 3.6kg .

TABLE 16

Gross pathological features in the abdominal cavity of aborted fetuses

Tissue	Description of lesion	Number of cases
Intestine and mesentery	congestion	6
	petechial haemorrhages	4
Mesenteric lymph glands	hyperplasia	2
Liver	enlargement	30
	congestion	24
	jaundice	2
	fibrinous peritonitis	2
Kidney	congestion	13
Bladder	mucosal surface haemorrhage	2
Adrenal gland	congestion	6
Spleen	splenomegaly	3
	sub-capsular haemorrhage	3

The descriptions of kidney and adrenal gland lesions were made from gross appearance and examination of the cut surfaces. Congestion was often observed at the boundary of cortex and medulla of both organs. This pattern of lesion often accompanied congestion, ecchymotic or petechial haemorrhages in liver, heart, and lungs, and was associated with generalised fetal septicaemia in 20 cases.

Two examples of ecchymotic or "paint brush" heamorrhages sited in the bladder mucosa were found (F3060; F3223),

along with similar lesions on splenic capsule and epicardium. Kidneys were also congested.Positive titres to L.hardjo were obtained from cows in these cases.

In one recorded case of splenomegaly (F1336), the spleen was 32cm long, 12cm wide, and weighed 2.2kg; the fetus itself weighed 32kg. The liver weighed 1.8kg and there was a moderate ascites.

h) Pathological findings in fetal thorax.

The lesions found in the thorax of aborted fetuses are listed in Table 17.

TABLE 17

Gross pathological features in the thoracic cavity of aborted fetuses

Tissue	Description of lesion	Number of cases
Heart	pallor	10
	epicardial petechial haemorrhage	18
	endocardial "paint brush" haemorrhage	2
	fibrinous pericarditis	4
Lung	oedema	33
	consolidation	4
	petechial haemorrhage	11
	fibrinous pleurisy	2
	frank haemorrhage in airways	1
	expanded airways	9
Thymus gland (thoracic)	petechial haemorrhages	2

The gross haemorrhage found in the trachea and bronchi of one fetus (F1949) stemmed from an organised, mixed

thrombus sited in the caudal vena cava, 4cm from the base of the heart. The vein appeared to have ruptured, causing massive haemorrhage into the right pleural cavity; blood was also present in the lung parenchyma. The liver of this fetus was enlarged but rather pale, but spleen appeared reduced in size with petechiation of its capsule.

Consolidation of fetal lungs occurred in apical, cardiac, and cranio-ventral diaphramatic lobes. Lesions found elsewhere in these abortuses were congestion and petechiation in intestine and heart; pure cultures of Gram +ve bacteria were made from fetal lungs and stomach contents in these cases.

Petechiae were frequently seen, either on the visceral pleural surface or in the parenchyma itself, and commonly in association with haemorrhagic spots on the epicardial surface. In four of these cases (F1387;F1389;F1438;F3223), a haemorrhagic placentitis was an additional finding.

A fibrinous pleurisy was found to be associated with lesions of pericarditis, and placental oedema, fibrosis, and placentitis. Bacillus licheniformis was isolated in pure culture from fetal stomach in all these cases (F1039;C1347; F2729) and one fetus was sero-positive for B.V.D as well (F1224).

Post-mortem lesions of cardiac haemorrhage were associated with a variety of lesions located elsewhere in a number of aborted fetuses, and a summary of these findings is given in Table 18.

TABLE 18

Gross pathological findings in 34 aborted fetuses which exhibited cardiac lesions, with suggested diagnoses

LESION	SUGGESTED DIAGNOSES (Number of fetuses)							
	B.lich	BVD	C.pyog	FDS	Fungal	IBR	L.hardjo	H.staph
Cardiac haemorrhage	1	3	2	1	1	3	3	2
Pericarditis	2	1	-	-	-	-	-	-
Lung haemorrhage	-	-	3	-	-	-	3	1
Lung congestion	-	-	3	1	3	-	2	-
Pleurisy	2	-	-	-	-	-	1	-
Liver congestion	-	-	-	1	3	-	2	-
Liver enlargement	-	5	-	-	-	-	4	-
Splenic haemorrhage	-	-	-	-	-	-	-	1
Intestinal haemorrhage	-	-	1	-	-	-	-	-
Urinary tract haemorrhage	-	-	-	-	-	-	2	-
Placental lesions	2	4	2	-	3	-	10	1
Total number of fetuses	4	5	3	1	3	3	13	2

Key to abbreviations:-

B. lich. - Bacillus licheniformis
BVD - Bovine Viral Diarrhoea virus
C.pyog - Corynebacterium pyogenes
FDS - Fetal Distress Syndrome
IBR - Infectious Bovine Rhinotracheitis
L. hardjo - Leptospira hardjo
H. staph - Haemolytic staphylococci

i) Pathological findings associated with no diagnosis.

Generalised autolysis of aborted fetuses was the largest single factor which contributed towards no diagnosis

being made after a full examination of all the available material. The gross lesions found in 48 fetuses into this category are set out in Table 19.

TABLE 19

Gross pathological lesions in 48 aborted fetuses where no diagnosis was made

No. of fetuses examined	Fetal lesion described
19	Autolysis
4	Fetal mummification
14	Lung oedema
1	congestion
2	haemorrhage
4	Heart petechiation
4	Liver enlargement
3	congestion
2	Splenic haemorrhage
1	Kidney congestion
1	Adrenal haemorrhage
	Placental lesion described
27	Necrosis
5	Oedema
2	Haemorrhage

4.2. Gross lesions of the fetal membranes.

Gross placental lesions were seen in either placentomes and/or chorio-allantoic membranes (C.A.M) of 66 abortions, the findings of which are summarised in Table 20. Comments on fetal membranes which were delivered with seven mummified fetuses have been excluded.

TABLE 20

Gross placental lesions found in 66 abortion episodes

Pathological findings	Placental tissue involved: Placentome	Cotyledon	Inter-cotyledonary CAM	Amnion
Necrosis	26	50	34	-
Oedema	3	-	12	6
Haemorrhage	6	7	3	1
Ulceration	-	-	5	-
Epithelialisation	-	-	-	2
Thickening	-	-	-	3
Adventitious placentation	-	-	4	-

Key CAM - Chorio - allantoic membrane

Pathological examination of fetal membranes was of limited diagnostic value because of several factors;

i) retention of fetal membranes (R.F.M) was a consistent clinical feature of all the abortion cases encountered, excluding the seven mummified fetuses. It was possible to remove only a relatively

small portion of the placenta from the uterus, and this was often inter-cotyledonary C.A.M.

ii) in a total of 87 cases, the cervix was not open sufficiently to enable manual removal of a placentome from the uterus.

iii) only single placentomes were manually removed from each aborted cow, in order to avoid any possible risk to the health of the dam following any more extensive procedure.

Gross lesions of the placentome were described after examination of the cut surface of the tissue. Necrosis was common, characterised by yellow discolouration and occasional haemorrhage within the pallisade zone, located between the attached chorio-allantois and the fibrous stalk of the caruncle. It was often very difficult to separate fetal placental tissue from the caruncle, despite the use of gentle traction, especially when oedema was present.

Necrosis of the inter-cotyledonary chorio-allantois was frequently seen; there was a loss of translucency of the membranes, which often took on an opaque, pinkish hue. In 13 cases, necrosis had progressed so far that the membranes were almost grey in colour. These changes almost certainly obscured any pathological changes that may have been present. The cotyledons attached to these membranes were similarly discoloured, but could also be dry and easily fragmented. Inter-cotyledonary portions of C.A.M were often oedematous and appeared thickened by a clear, gelatinous

fluid. Where necrotic change had also occurred, the layer of chorionic epithelium in contact with the endometrium was often discoloured and accumulations of reddish-brown to yellow exudate were found, together with obvious membrane congestion. Occasionally multiple small focal lesions, consisting of raised areas of epithelial cells occurred on chorionic epithelium adjacent to the endometrium. The edges of the lesion were discretely raised, thickened, slightly yellow in colour, and surrounded by a circular area of congestion; its centre was crater-like. The lesions could coalesce. Other occasional lesions in this portion of tissue were ecchymoses or petechiae within the connective tissue stroma.

Adventitious placentation was found in four cases (F962;F998;F1276;F1387), as numerous small islands of villous cotyledonary tissue erupting from inter-cotyledonary C.A.M. Whilst individual areas involved were usually not larger than 4cm diameter, much of the C.A.M could be involved; they were without regular shape. No other pathological features were consistently related to these observations.

4.3. **Immunological findings**.

a) Fetal immunoglobulin and serological results.

Thoracic fluid samples recovered from 35 of 136 fetuses examined post mortem were positive for the presence of immunoglobulins IgG and IgM. Histo-pathological lesions were recorded in fetal or placental tissues in 28/35 cases.

Of these 35 fetuses, 13 gave a positive result for serum neutralising antibodies to B.V.D virus. 5/35 gave a positive micro-agglutination test for L.hardjo with titres of up to 1/640, and two additional fetuses were F.A.T. positive to 1/50 (C130;F823), but there was no corroborative evidence to support such a diagnosis from other materials submitted for investigation. In five abortuses which gave a negative result for of IgG and IgM, the ELISA method of identifying I.B.R antibody proved positive.

b) Maternal serological results associated with abortions.

In paired blood samples obtained from 149 cattle, 62 showed either changing or high antibody titres to B.V.D virus (see Table 21), I.B.R virus (see Table 22), or L.hardjo (see Table 23). The criteria which were used to judge the significance of each result are given in each case.

Of eleven cases in which rising or falling titres were demonstrated to I.B.R virus, no fetal immunological response was exhibited by either radial diffusion or ELISA techniques.

TABLE 21

Numbers of cows and their serological titres to BVD virus, in the presence or absence of significant fetal or placental lesions

Serological result	Lesions	Maternal serology titres (SN) at 1st sampling >128	32-64	2-16	0	Total
POSITIVE (Four-fold change in titre)	Present	11	10	-	5*	26
NEGATIVE (Less than four-fold change in titre)	Present	-	14	19	10	43
POSITIVE	Absent	6	2	3	2*	13
NEGATIVE	Absent	-	7	11	49	67

* Based on fetal serology
SN - Serum neutralisation

TABLE 22

Number of cows and their serological titres to IBR virus, in the presence or absence of significant fetal or placental lesions

Serological result	Lesions	Maternal serology at 1st sampling (O.D. by EIA) > 1.00	0.2 - 0.99	0 - 0.19	Total
POSITIVE (Greater than 0.2 change in OD)	Present	6	6	1 + 1*	14
NEGATIVE (Less than 0.2 change in OD)	Present	-	3	-	3
POSITIVE	Absent	5	2	-	7
NEGATIVE	Absent	2	5	118	125

OD = Optical Density
EIA = Enzyme Immuno-assay
* = Based on fetal serology

TABLE 23

Numbers of cows and their serological titres to L. hardjo in the presence or absence of significant fetal or placental lesions

serological result	Lesions	Initial titres to L. hardjo < 1/100	1/400	1/1600	Total
POSITIVE Titres > 1/400	Present	-	7	6	13
NEGATIVE Titres < 1/100	Present	5	-	-	5
POSITIVE	Absent	-	4	1	5
NEGATIVE	Absent	126	-	-	126

Histological lesions were demonstrated in aborted material from 91 of the cases examined, but a diagnosis was made in only 57 of these.

4.4. Histopathological lesions in aborted fetuses.

Lesions were observed in 57 fetuses and their placental tissues where a likely association with an infectious cause for the abortion could be made. Lesions were found in similar material from 56 other cases were no such link could be made through lack of supportive evidence. In one case of "Fetal Distress Syndrome", microscopic lesions could be identified without further laboratory aids to diagnosis.

In abortion material obtained from 9/56 cases to which no diagnosis was attached, histological lesions suggested a likely diagnosis which could not be supported by laboratory techniques currently in use at the V.I.Centre, Penrith.

A comparison is made in Figure 4-3 of the fetal lesions found in cases where a possible diagnosis was made, against those where no diagnosis was reached.

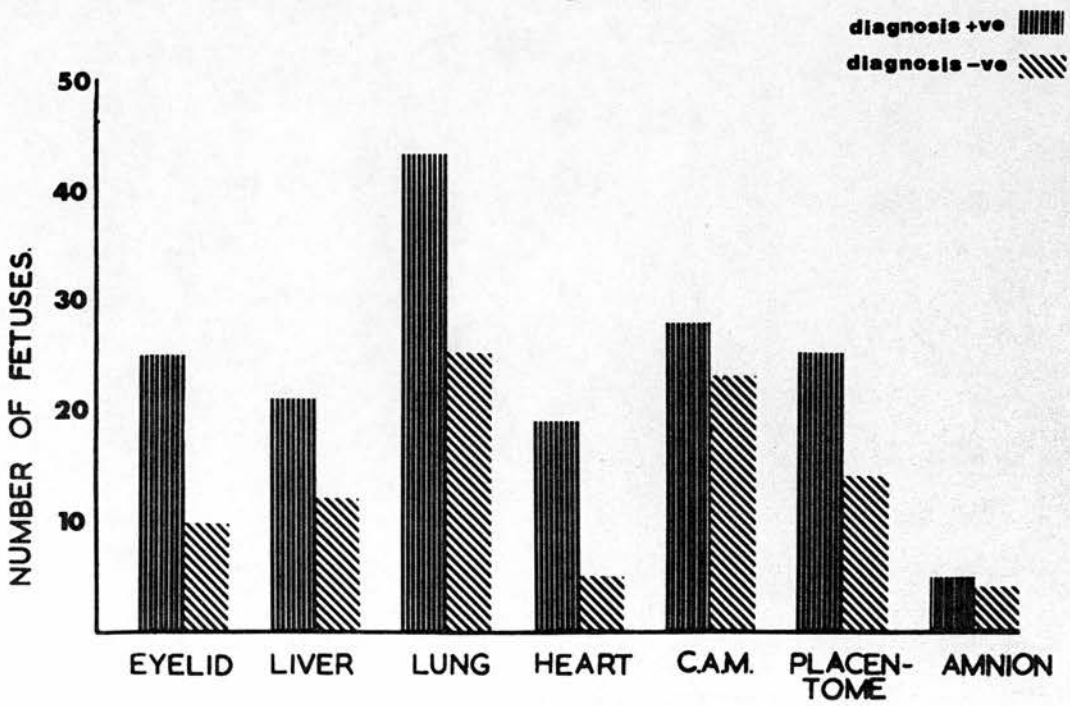

Fig. 4-3 **Distribution of histopathological lesions found in tissues of 136 aborted fetuses, from which a diagnosis was suggested for 57 cases.**

A description now follows of the lesions found in particular tissues.

a) Lesions of fetal eyelid.

Demonstrable lesions were found in 34/132 sections of fetal eyelid examined. Sections were cut at right angles to the muco-cutaneous junction in order to display 2-3mm of keratinised skin, the same length of conjunctival mucous membrane, and the supporting connective tissue between.

Sections taken from tissue of healthy fetuses showed normal histological features of skin, hair follicles, and the tarsal gland. Also included was palpebral conjunctiva which was composed of a single layer of cuboidal cells, without any mucus-producing cells present, at this site. The epithelium is supported by connective tissue within which numerous small blood vessels and capillaries are located (Fig 4-4).

Histological lesions were able to support a diagnosis being made in 26/34 cases and these, together with a summary of the all the results, are set out in Table 24.

Most of the lesions consisted of inflammatory cell infiltrations in the sub-epithelial connective tissue (Fig 4-5), with occasional focal aggregations of neutrophils. In two cases, fungal hyphae were found adjacent to the skin and conjunctival surface, and there was evidence of an associated folliculitis (Figs 4-6;4-7).

TABLE 24

The distribution and description of histological lesions in eyelid, with concurrent serological and microbiological findings in 34 aborted fetuses.

Fetus number	Crown rump cm	Fetal (+)& maternal (x) serology IgG	IgM	IBR	BVD	L.hdjo	Micro-biology	Description of lesions MNI	PNI	Epith hyper	Hyphae
F 017	82	-	-	-	-	-	C pyogenes	-	+	-	-
F 468	60	+	-	-	-	-	N.H.coli	+	-	-	-
F 558	66	-	x	x	-	-	N.H.coli	+	-	-	-
F 785	63	-	x	-	+	-	C.pyogenes	+	-	-	-
F 886	70	-	-	-	+x	-	H.coli	+	+	-	-
F 888	66	-	+	-	-	-	H.streps.	+	+	-	-
F 962	86	-	-	-	-	-	mixed cells	+	+	+	-
F 988	70	-	-	-	-	-	mixed cells	+	+	-	-
F 997	66	-	-	x	-	-	C.pyogenes	+	+	-	-
F1011	66	+	+	-	-	-	mixed cells	+	-	+	-
F1224	80	-	-	+	x	-	mixed cells	+	-	+	-
F1219	49	-	x	-	-	-	B.lich formis	-	+	-	-
F1347	61	+	-	-	-	-	B.lich formis	+	+	+	-
F1358	79	-	x	-	-	-	H.coli	+	+	-	-
F1357	69	-	-	x	+x	-	mixed cells	-	+	-	-
F1389	83	-	-	x	-	-	mixed cells	+	-	-	-
F1438	36	+	-	+	x	-	mixed cells	+	+	-	-
F1462	78	-	-	x	-	-	mixed cells	+	-	+	-
F1492	90	-	-	-	-	-	mixed cells	+	-	+	-
F1505	68	+	-	x+	-	-	mixed cells	+	+	-	-
F1580	81	+	-	-	-	-	H.staphs	-	+	-	-
F1599	92	+	-	x+	-	-	H.coli	-	+	-	-
F1720	46	+	-	-	-	-	B.lich formis	+	+	-	-
F1721	90	-	-	-	-	-	mixed cells	+	+	+	-
F1795	75	-	-	-	-	-	C.pyogenes	+	+	+	-
F1981	56	+	-	-	-	-	fungi	-	+	-	+
F2000	84	-	-	-	-	-	mixed cells	+	-	+	-
F2526	64	-	-	-	-	-	S.typh murium	-	+	-	-
F2589	55	+	-	-	-	-	mixed cells	-	+	-	-
F2591	76	+	-	x+	-	-	mixed cells	+	+	-	-
F2879	79	-	-	-	-	-	mixed cells	-	-	-	+
F2915	68	-	-	x	-	-	H.coli	+	+	-	-
F2984	74	+	-	x	x+	-	mixed cells	+	+	-	-
F3023	58	-	-	-	-	-	fungi	+	+	-	+
F3078	46	-	-	-	-	-	mixed cells	+	-	-	-

Key to abbreviations :-

MNI - Mononuclear cell infiltration
PNI - Polymorphonuclear cell infiltration
Hyphae - fungal hyphae present
Epith hyper - Epithelial hyperplasia

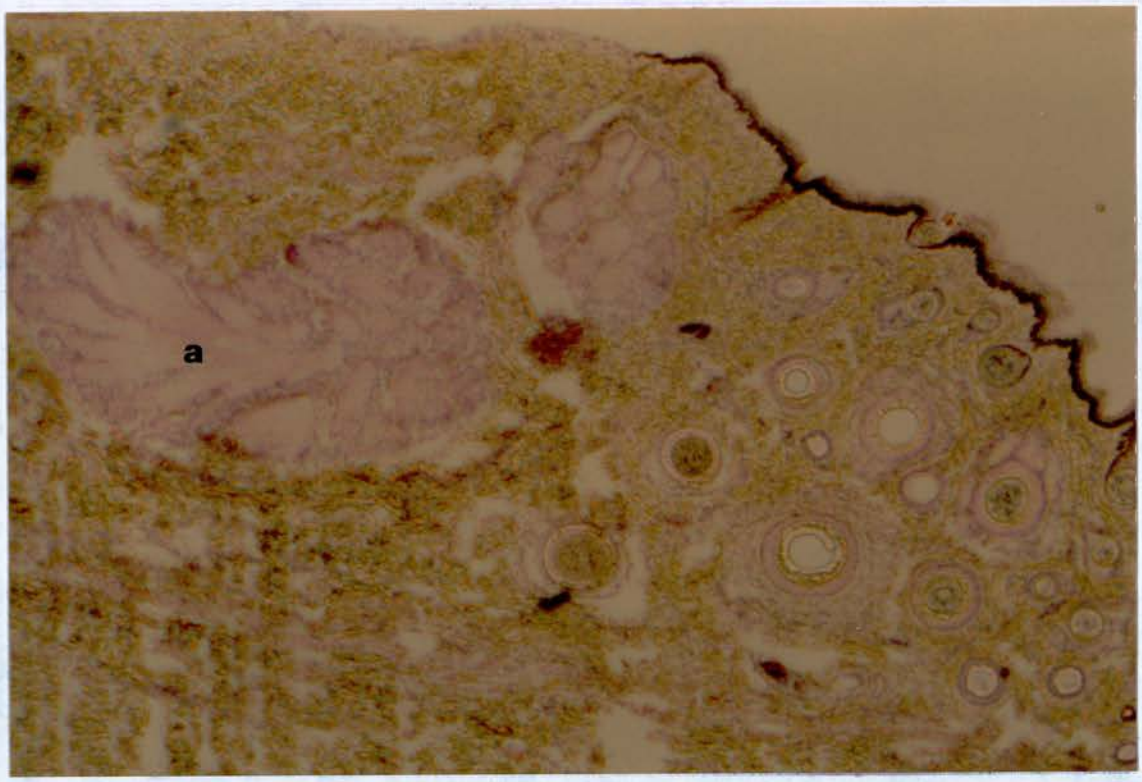

Fig 4-4 Eyelid F559; skin of the eyelid is continued by mucous membrane (palpebral conjunctiva). The tarsal gland (a) is prominent. x60

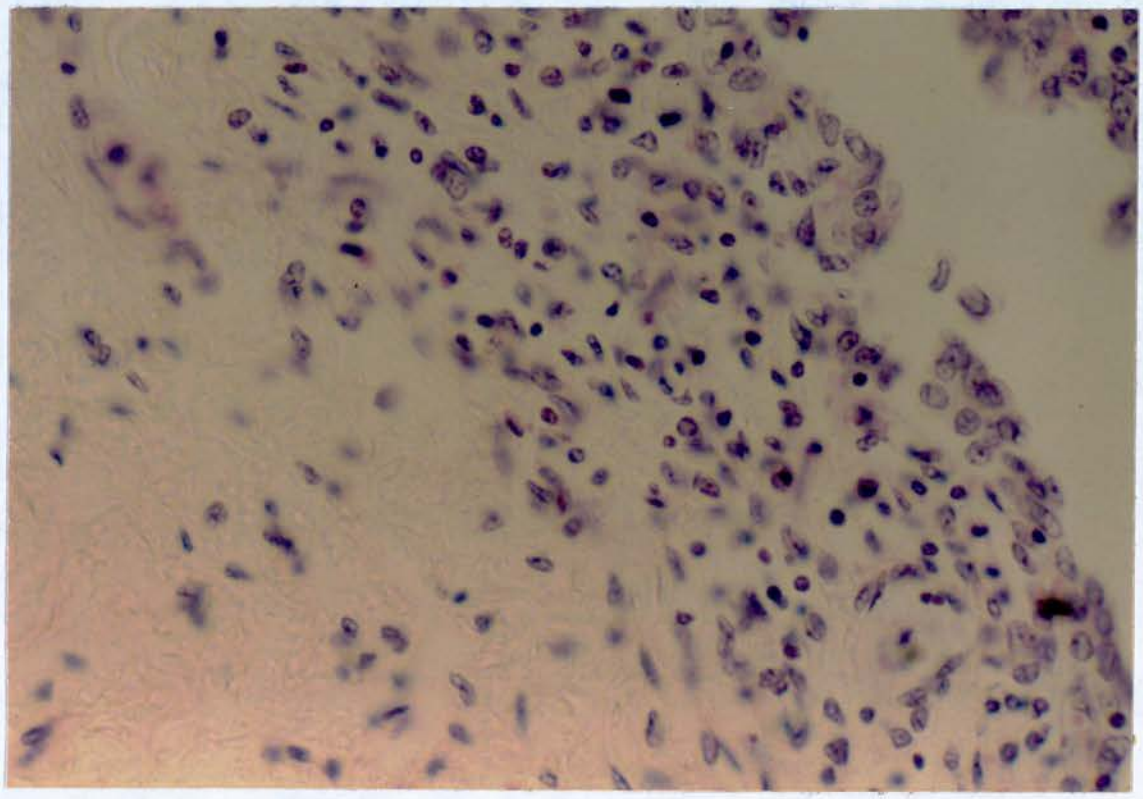

Fig. 4-5 Eyelid F2915; diffuse mononuclear inflammatory cell infiltration in sub-conjunctival tissues with some congestion. x540

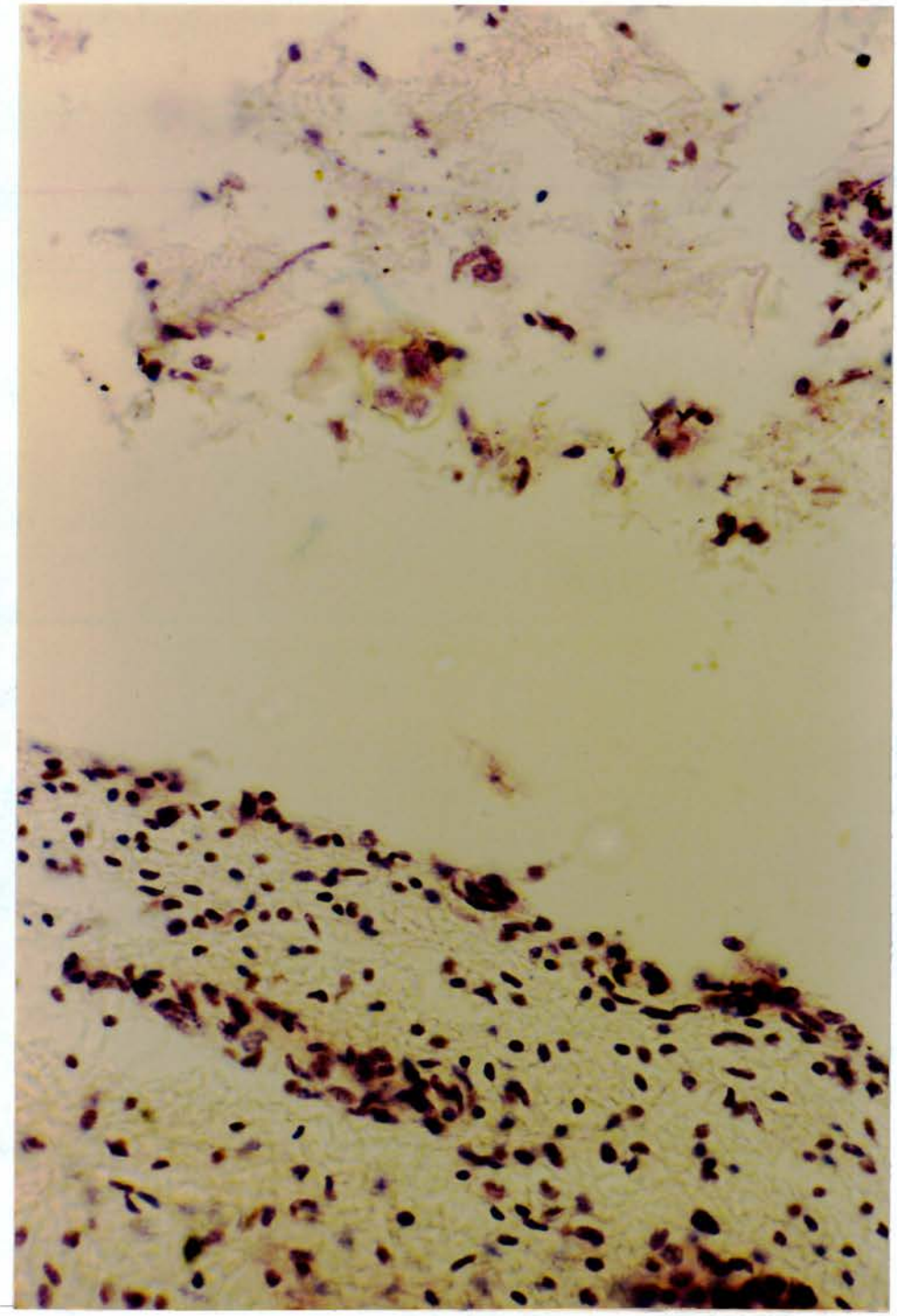

Fig. 4-6 Eyelid F3023; fungal elements, neutrophils and amniotic debris adjacent to palpebral conjunctiva with a sub-conjunctival polymorphonuclear inflammatory cell infiltration. x 540

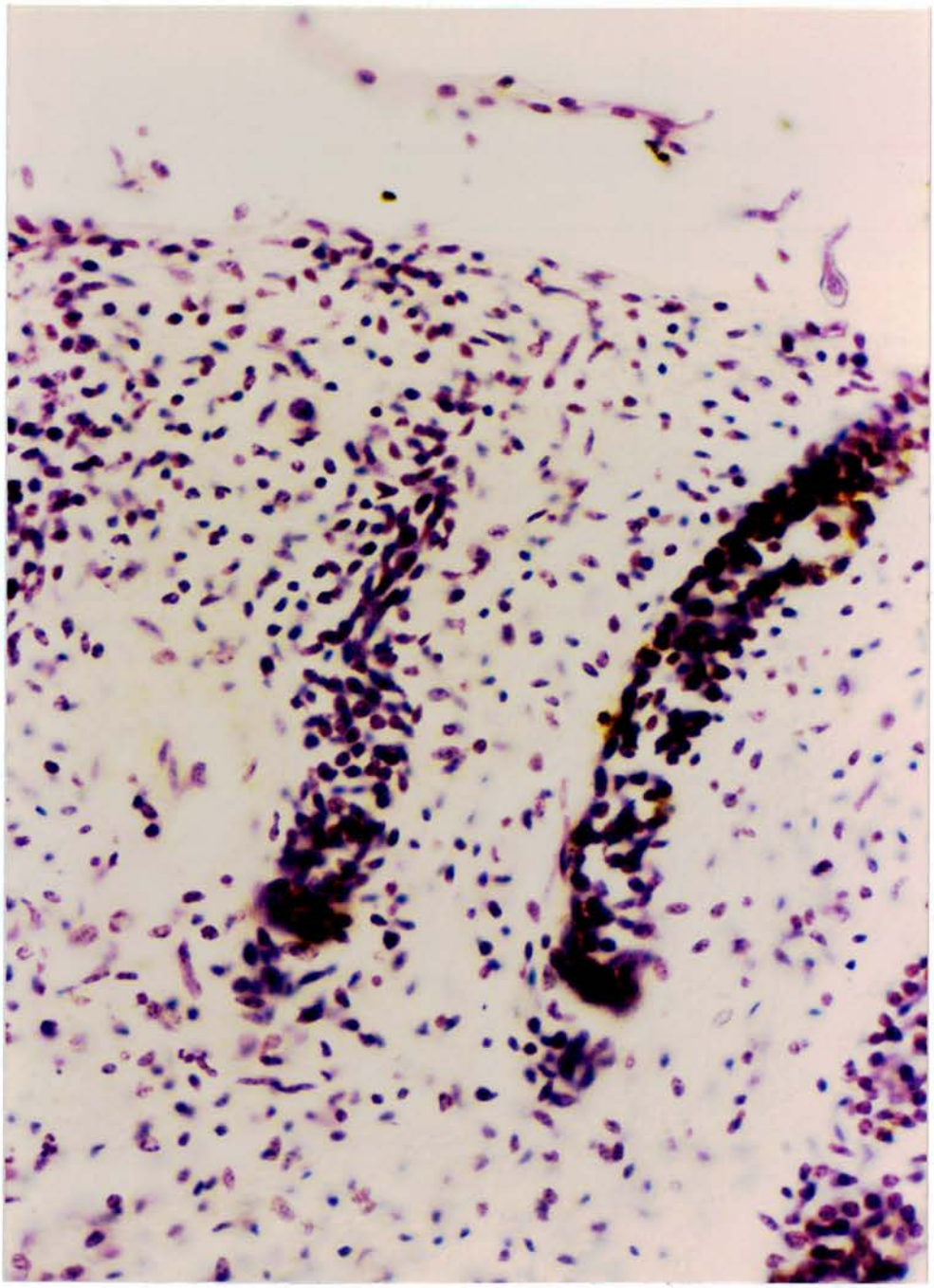

Fig. 4-7 Eyelid F3023; fungal elements at muco-cutaneous junction, accompanied by folliculitis; moderate polymorphonuclear cell infiltration in superficial layers of epidermis. x540

Hyperplasia of palpebral conjunctiva was found in 9/34 sections examined. Low cuboidal epithelium had changed to a stratified form, and large, mucus-producing goblet cells were located within this accumulation of cells. The degree of severity of these changes varied from extensive epithelial hyperplasia with few goblet cells in evidence (Fig 4-8), to focal lesions showing mild stratification with numerous goblet cells present (Fig 4-9). A moderate infiltration of mixed inflammatory cells in the sub-conjunctival tissue accompanied this lesion in 8/9 cases.

A coexisting placentitis was apparent in 6/9 of these cases, characterised by focal villous necrosis with a related heavy mixed inflammatory cell infiltration. In two

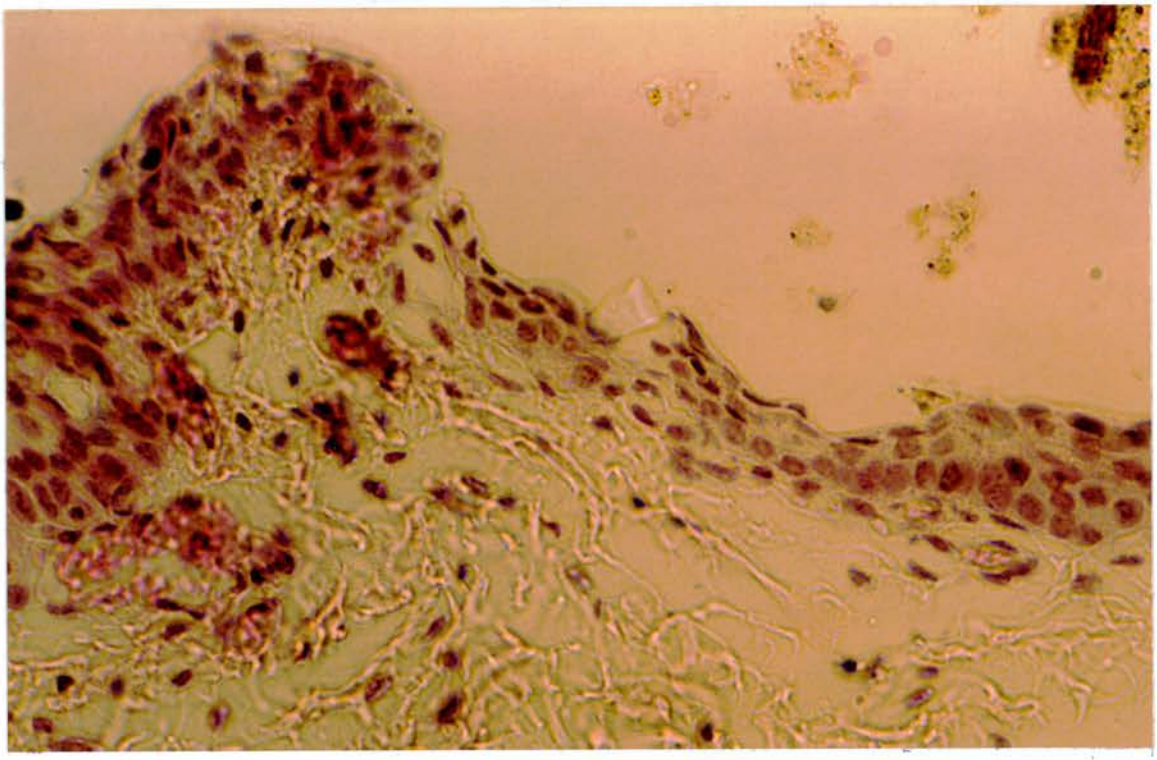

Fig. 4-8 **Conjunctiva F1492; extensive epithelial hyperplasia and pseudo-stratification, enclosing mucous producing cells; also present are focal aggregates of polymorphonuclear inflammatory cells in sub-conjunctival tissue.** x540

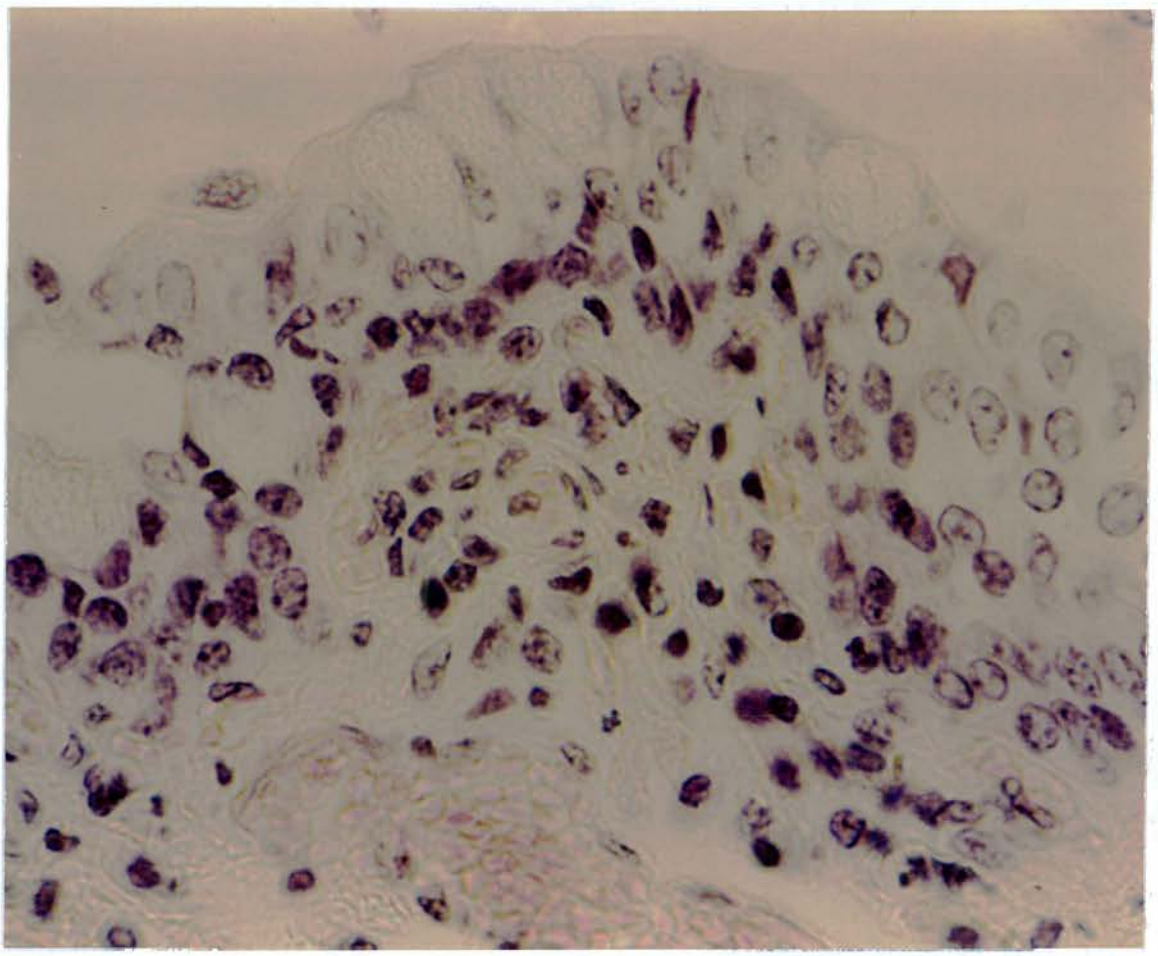

Fig. 4-9 **Conjunctiva F1224; hyperplasia and goblet cells in conjunctival epithilium accompanied by moderate polymorphonuclear imflammatory cell infiltration,x864**

cases (F962; F1462), an amnionitis was present, characterised by focal mineralisation, fibrosis, polymorph and mononuclear cell accumulations. Immunoglobulin was demonstrable in only 2/9 fetuses which presented this pathological profile, and there were no microbiological or serological findings consistently related to these cases.

b) Fetal heart lesions.

The histological structure of fetal heart tissue does not appear to differ significantly from that found in neonatal or other young cattle. Because of the relatively narrow width of the myocardium of the right ventricle, routine sections of heart muscle were usually obtained from this site, so that both epicardial and endocardial surfaces could be examined on the same slide.

Lesions were found in 26/134 fetuses examined, and consisted mainly of mixed inflammatory cell infiltrations in perivascular areas of the myocardium. A primarily mononuclear response was found in 16 of these 26 cases, 11 of which could be associated with a possible infectious cause of abortion.

Lesions of non-specific myocarditis were found in 18/26 cases, characterised by focal areas of degenerative or necrotic change which included swelling and loss of striation in myocardial fibres. Lesions could be surrounded by mononuclear inflammatory cells with the occasional presence of polymorphonuclear cells. In 5/18 cases, the lesions were more severe and widespread; areas of focal necrosis were surrounded and infiltrated by mixed inflammatory cells, which could extend to the interstitial connective tissue (Fig 4-10). All these cases had coexisting lesions of necrotic placentitis, and a serological response to I.B.R was also associated with the lesion.

In a further 6/18 cases showing lesions of myocarditis

there was serological evidence of an active B.V.D viral infection, but in only half of these cases were there any placental lesions present. Of these, abortions F1337 and F2984 gave evidence of an accompanying acute infection with L.hardjo. A summary of the pathological findings is given in Table 25.

TABLE 25

Microscopic lesions in hearts of 26 aborted fetuses, showing related serological and microbiological findings.

Fetus No.	Crown rmp cm	Fetal (+) & maternal (x) serology IgG IgM	IBR	BVD	L. hdjo	Micro-biol	Description of lesions H'rrge	Degen	Nec.	Peri cuff	MNI	PNI
F 004	85	-	-	+	-	N.H.coli	-	+	-	-	+	-
F 028	68	-	-	x+	-	mixed cells	-	-	-	+	+	-
F123	91	+	+	-	-	mixed cells	+	+	-	-	-	-
F 160	93	-	-	-	-	H.coli	-	-	+	-	+	+
F 239	90	-	-	-	-	mixed cells	-	-	-	-	+	-
F 558	66	-	-	-	-	N.H.coli	-	-	-	-	+	-
F 785	63	-	x	-	+	C.pyogenes	-	-	-	-	+	-
F 797	50	-	-	-	-	N.H.coli	-	-	+	-	+	-
F 799	62	-	-	-	-	C.pyogenes	-	-	+	-	-	-
F 886	70	-	-	-	x+	H.coli	-	-	-	-	+	-
F 889	75	-	x	-	-	C.pyogenes	-	-	+	-	+	-
F 997	66	-	-	x	-	C.pyogenes	-	+	-	-	+	-
F1221	88	-	x	-	-	mixed cells	-	-	+	-	-	+
F1224	80	-	-	x	-	mixed cells	-	-	-	+	-	+
F1251	94	-	-	-	-	B.lich'formis	+	-	-	-	-	-
F1337	67	+	-	x	x	mixed cells	-	+	-	-	-	+
F1358	79	-	x	-	-	H.coli	-	+	+	-	-	-
F1387	69	-	x	-	x+	mixed cells	-	-	+	-	+	+
F1388	68	-	-	-	-	mixed cells	-	+	-	-	+	-
F1438	36	+	-	+	x	mixed cells	-	+	+	-	+	+
F1452	50	+	-	+	-	H.coli	-	+	+	-	+	+
F1462	78	-	x	-	-	mixed cells	-	+	-	-	-	+
F1599	92	+	-	x+	-	H.coli	-	+	+	-	+	-
F1879	76	-	x	-	-	mixed cells	-	-	+	-	-	+
F2984	74	+	-	x	x+	mixed cells	-	+	-	-	+	-

Key to abbreviations :-

H'rrge - Haemorrhage
Degen - Degenerative change
Nec. - Necrosis
Peri cuff - Perivascular cuffing
MNI - Mononuclear cell infiltration
PNI - Polymorphonuclear cell infiltration

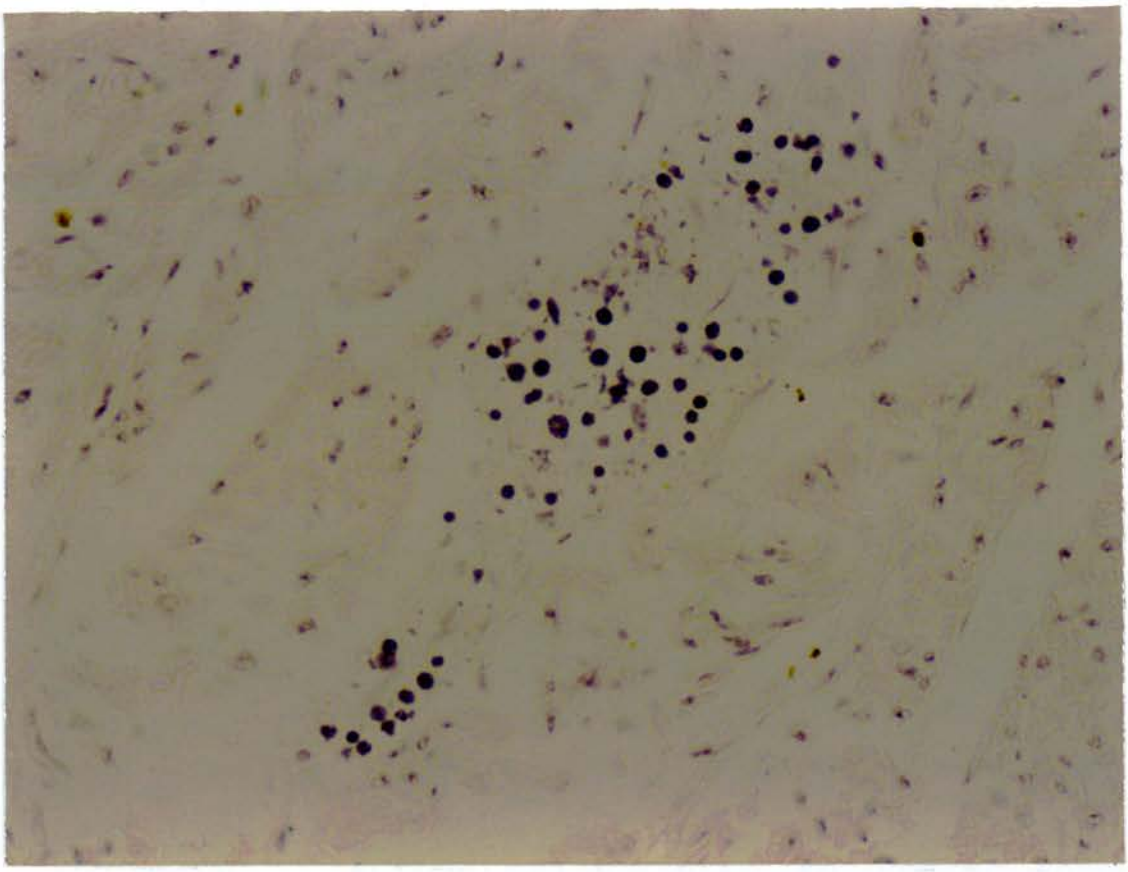

Fig. 4-10 **Heart F1600; multiple focal areas of myocardial necrosis with mononuclear inflammatory cell infiltration . Myocardium undergoing early autolysis.x864**

c) Lesions in fetal lungs.

The normal histological picture of lungs examined in this investigation varied with the fetal age, and is described as follows:

i) canalicular phase. Related to fetuses between 110 and 160 days gestation. The calibre of the peripheral airway is wide, and these developing bronchioles make up most of the lung parenchyma. The epithelial lining is made up of low columnar to cuboidal cells. Small blood capillaries are in close contact with this epithelium (Fig 4-11).

ii) terminal sac phase. Found in fetuses aged 160 days to

full term. The lining epithelium starts to differentiate into type I and type II pneumocytes. The branching of respiratory bronchioles becomes more complex with formation of terminal sacs and alveoli. The connective tissue elastic fibres which support these structures are prominent around air ways and within alveolar septae (Fig 4-12).

Histological lesions were identified in lungs from 65 fetuses. In 43/65 of these, a predominantly polymorphonuclear inflammatory cell infiltration of the lung parenchyma was present, associated with a bronchial pneumonia in 24 instances. In 13/65 of the remaining cases, inflammatory cell infiltration of interstitial tissue was made up of mononuclear cells, with occasional plasma cells and neutrophils present.

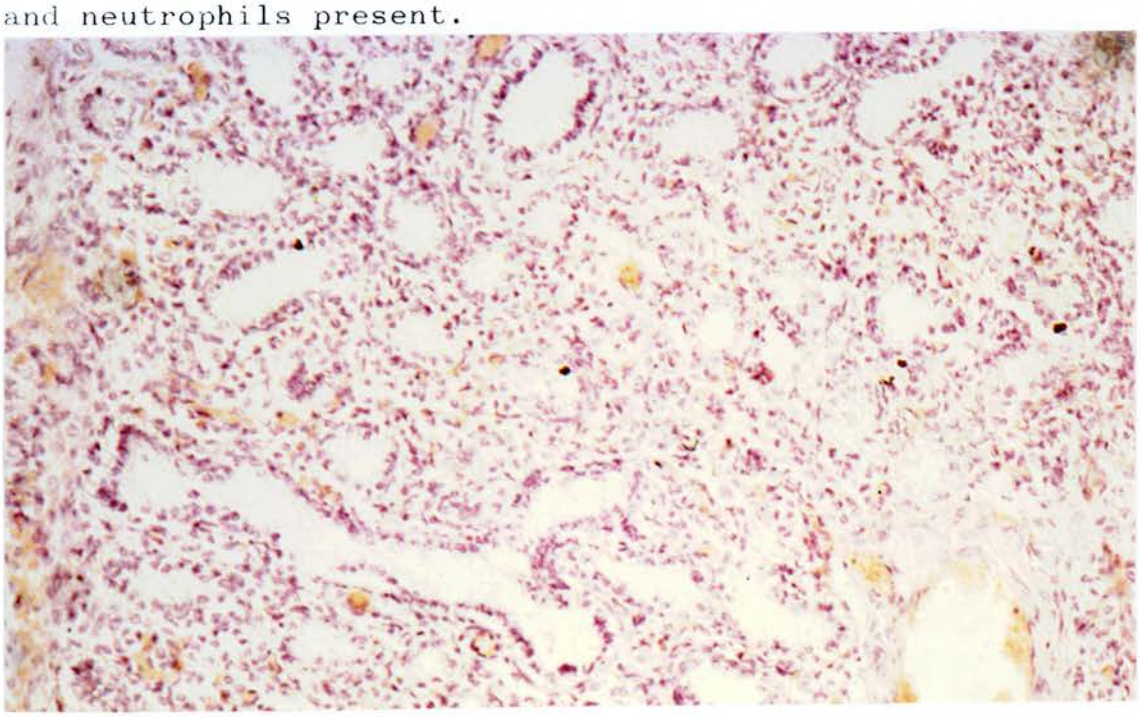

Fig 4-11 **Fetal lung; canalicular phase. Fetus C/R length 25cm. Low columnar to cuboidal epithelium, lining enlarged peripheral airways and surrounded by condensing mesenchyme. x212**

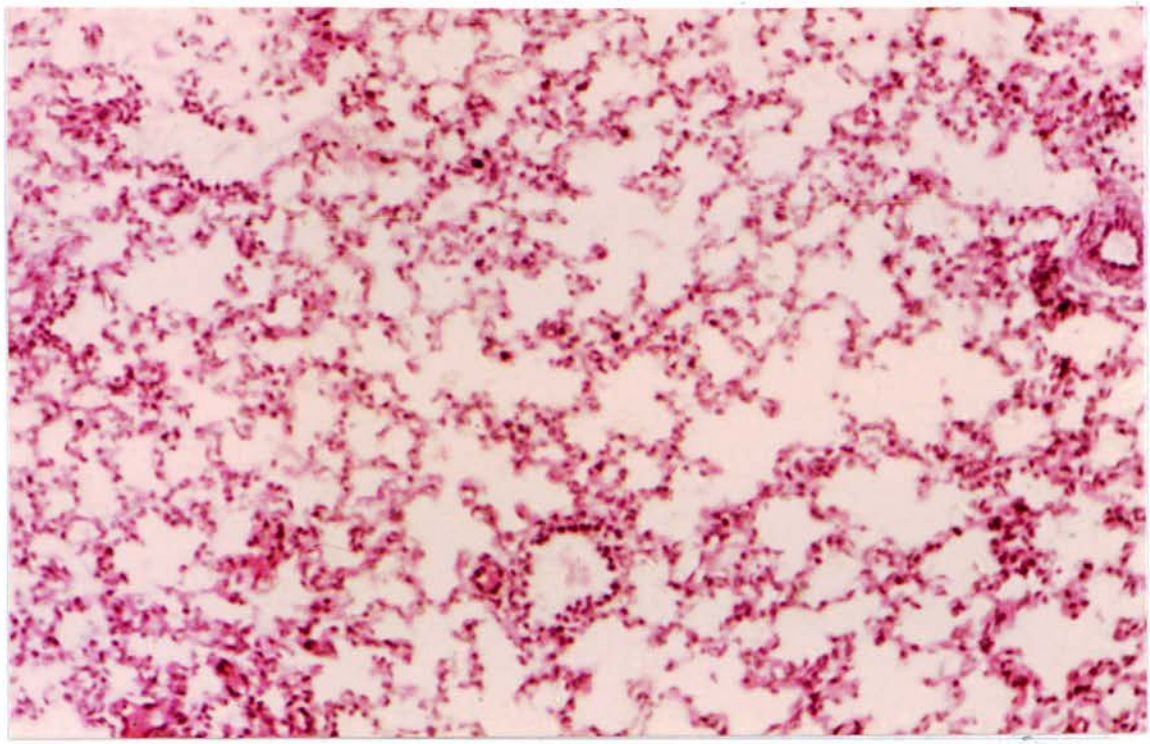

Fig. 4-12 **Lung, terminal sac phase (partially inflated with fixative). Progressive branching of bronchioles and terminal sac complexity. Differentiation of the lining epithelium is taking place with pneumocytes present.x212**

Of the cases in which an inflammatory cell infiltration was present, there were coexisting lesions in sections of other fetal tissues from eight abortuses; in eyelid there was epithelial hyperplasia and goblet cell formation, in cotyledon a villitis, and in amnion an amnionitis.

In the 13 lungs where there was a mainly mononuclear cell infiltration, the lesion was associated with positive serological evidence of I.B.R.(Fig.4-13), B.V.D., and L.hardjo (Fig.4-14) infections, six of the cases having a possible multiple aetiology.

A polymorphonuclear inflammatory cell infiltration around bronchi and alveoli in the presence of inflammatory exudate, necrotic debris, and bacteria in the airways was

observed in 24/64 fetal lungs. In 15/24 of these cases, pure bacterial cultures were grown from lung tissue, C.pyogenes (Fig.4-15), B.licheniformis,haemolytic E.coli, haemolytic H.Staphylococci, and fungi being the organisms most commonly isolated. Histological evidence of a concurrent placentitis was found, either in cotyledon or placentome. Of the remaining cases which presented this feature, mixed bacterial cultures were grown where no single pathogen could be implicated. Haemolytic Staphylococci and Streptococci were frequently isolated, and serological evidence of I.B.R infection was found in four of these latter cases (F1011; F1032; F1219; F1580).

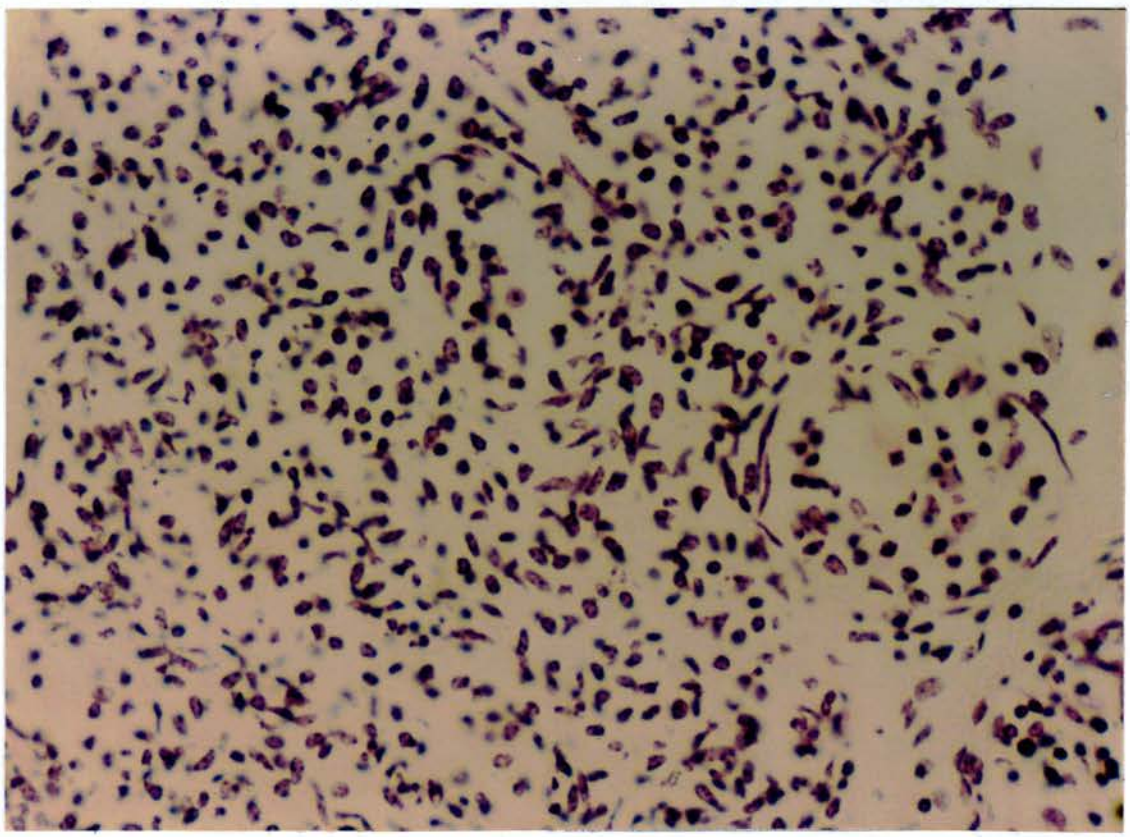

Fig. 4-13 **Lung C1958; interstitial infiltration of predominately mononuclear inflammatory cells. x 540**

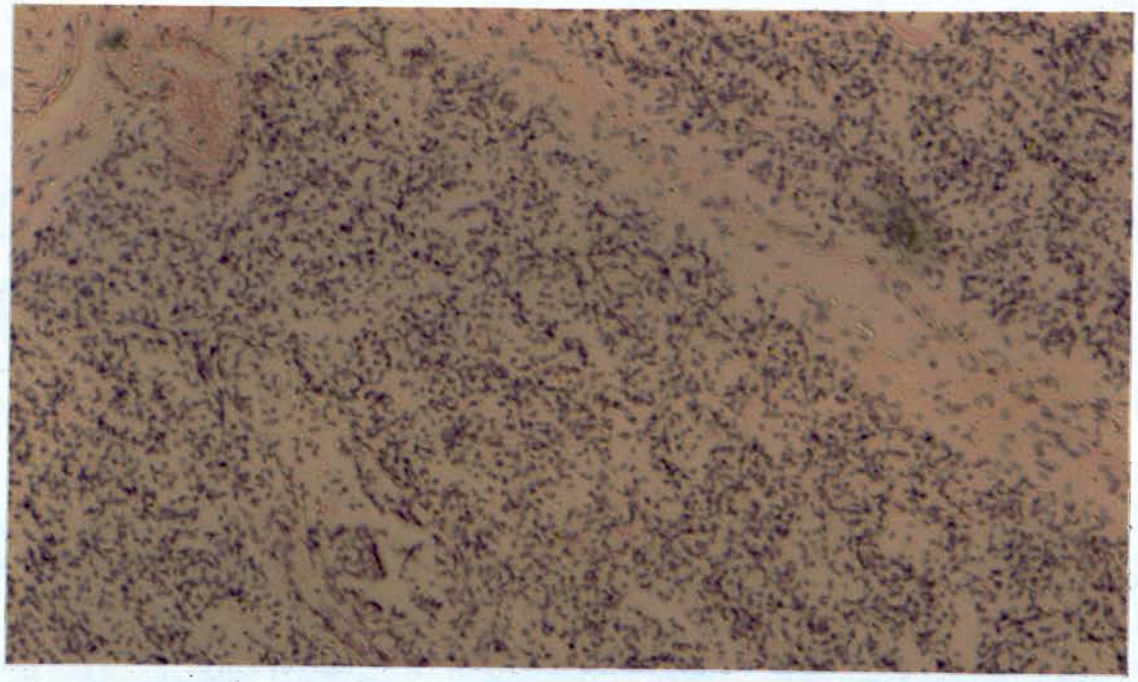

Fig. 4-14 Lung F1600; moderate interstitial inflammatory cell infiltration, comprising mainly of mononuclear cells. A few have infiltrated the inter-lobular connective tissue. Some congestion is present. x 212

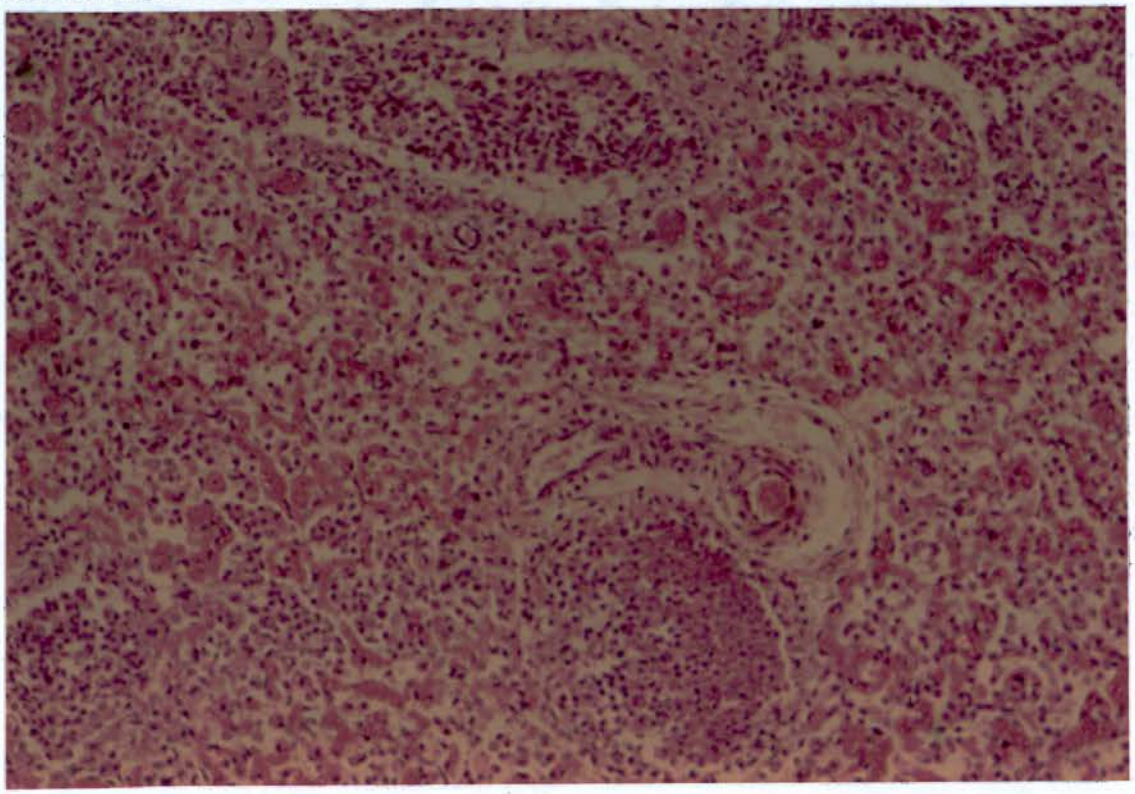

Fig. 4-15 Lung F017; acute bronchitis and broncho-pneumonia. Some airways are filled with exudate. Mixed inflammatory cell infiltration present in surrounding peribronchiolar tissue.x 212

Congestion in inter-alveolar tissue was a feature of 22 of the lung sections examined, often accompanied by lesions in other tissues.In eight of these cases (F123; F160; F239; F1224; F1358; F1387; F1462; F1599), heart lesions were also present together with serological evidence of either B.V.D or I.B.R infection in fetus or aborted dam. Erythrocytes were packed into blood capillaries adjacent to developing airways and inter-alveolar septa (Fig.4-16); inter-lobular congestion and haemorrhage was variably present. Interstitial fibrosis was found in five of these sections, where the larger pulmonary blood vessels could be packed with erythrocytes and some leucocytes.

Inter-lobular oedema was occasionally found, with or without fibrocyte infiltration into the connective tissue (Fig.4-17).

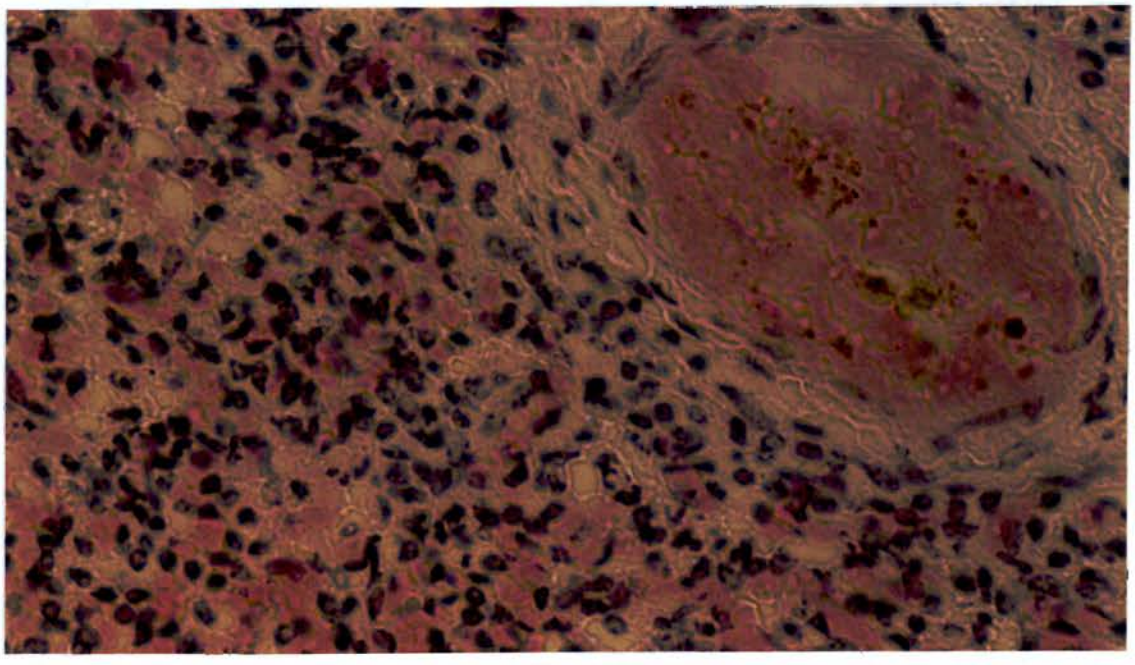

Fig. 4-16 **Lung F887; congestion of interstitial tissue, with alveolar capillaries engorged with erythrocytes. A small vein is distended with blood. Mixed, predominately mononuclear inflammatory cell infiltration present.x 540**

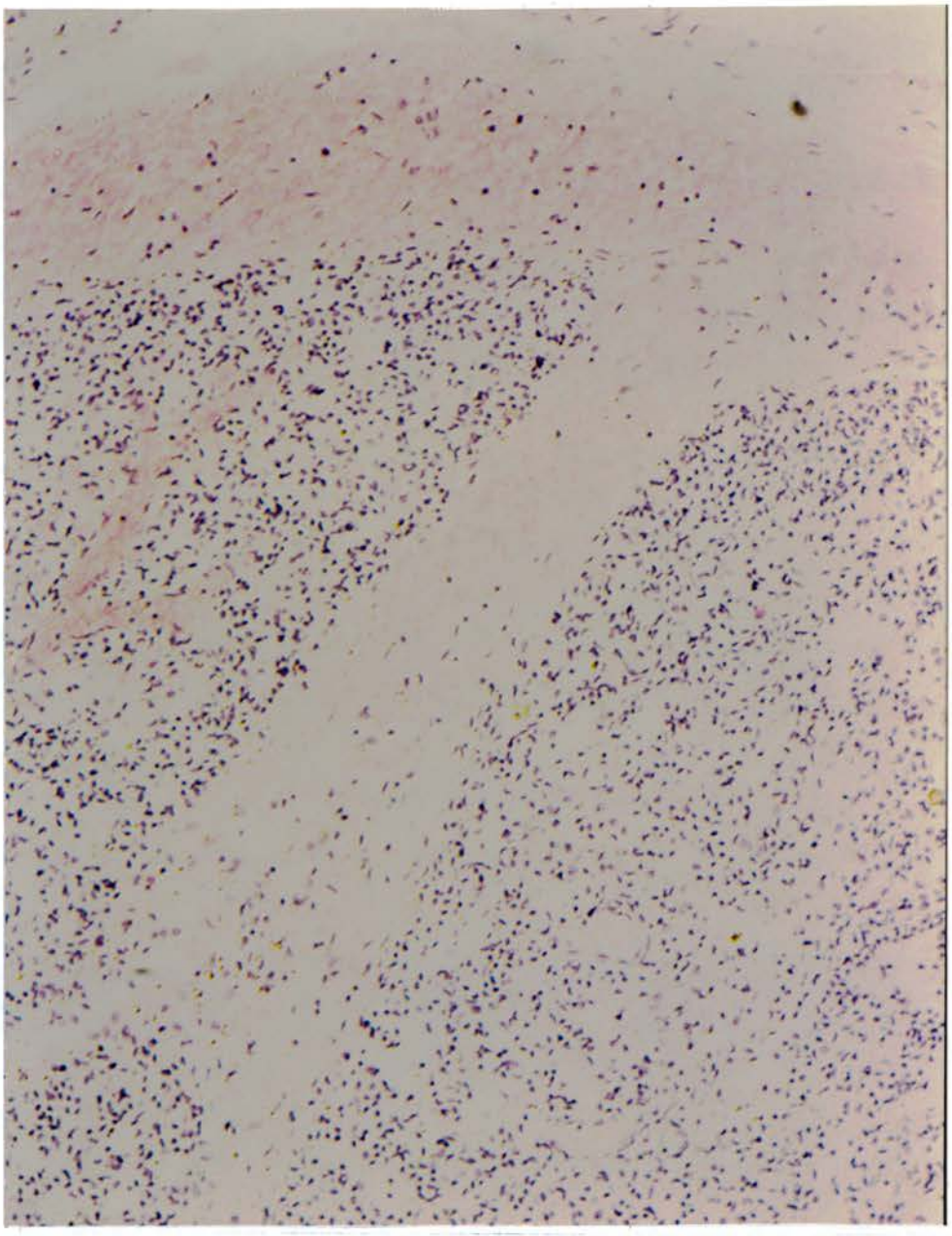

Fig. 4-17 **Lung F1387; sub-pleural haemorrhage and congestion. Inter-lobular oedema is present together with a few fibrocytes in the connective tissue. Mild mononuclear inflammatory cell infiltration is present in interstitial tissue, together with patchy congestion. x 212**

Inhalation of amniotic debris into fetal airways was found in 43/65 of lung sections. Meconium stained up a dull orange or brown colour with H & E stain; skin squamous cells were dense pink in colour with an irregular outline which did not usually contain a nucleus(Figs.4-18;4-19).

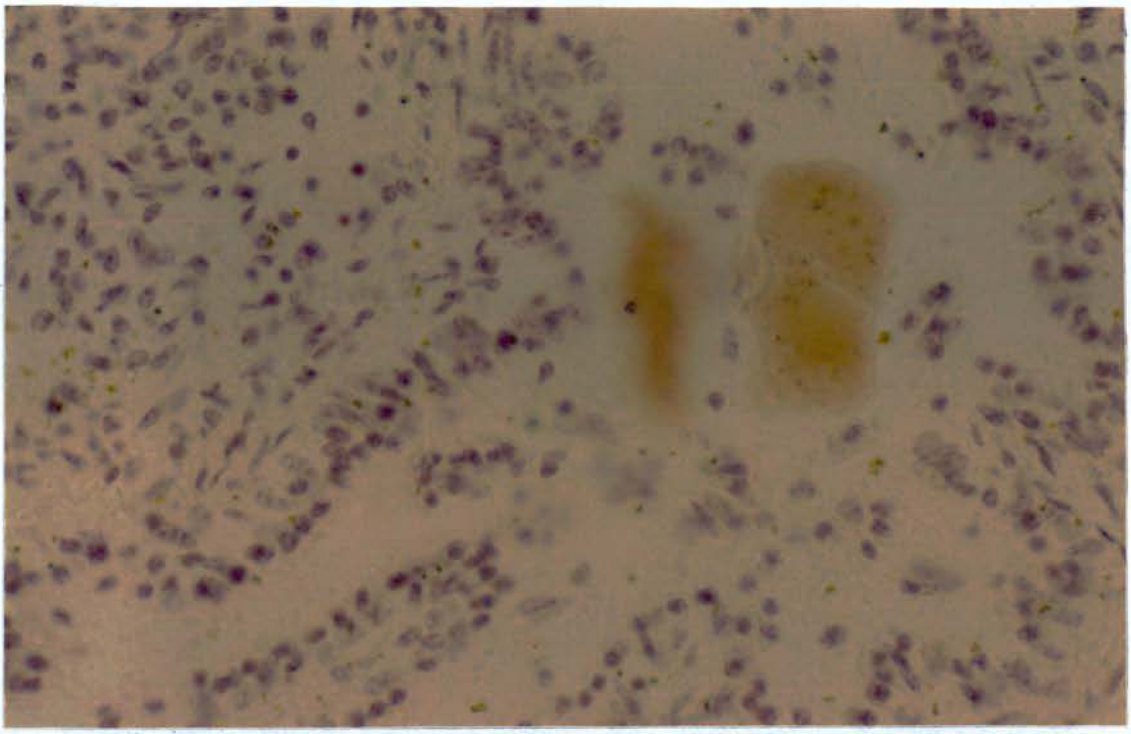

Fig. 4-18 Lung F2733; brown-staining meconium in large airway, without any accompanying inflammatory change.x540

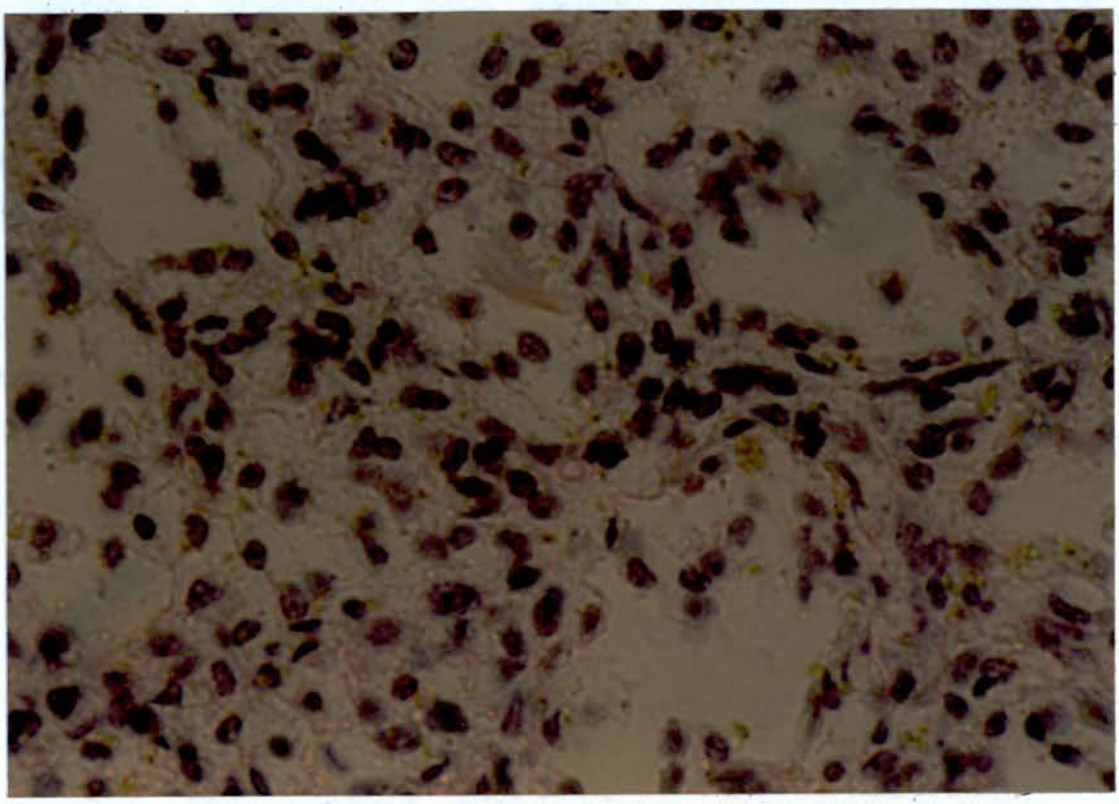

Fig. 4-19 Lung F972; squamous epithelial cell in airway with accompanying pneumonitis. Mononuclear inflammatory cells can be seen infiltrating the interstitial tissue. x 864

4/43 cases presented this pathological picture, which included a little interstitial congestion and oedema, but no inflammatory lesions were found elsewhere. No association with an infectious agent could be made with these abortions. Gross haemorrhages on epicardium and splenic capsule, with congestion and engorgement of the liver and the boundary of cortex and medulla of the kidneys were confirmed histologically e.g. F1492; F2394 (Fig.4-20). "Fetal distress syndrome" was the diagnosis attached to these and similar cases.

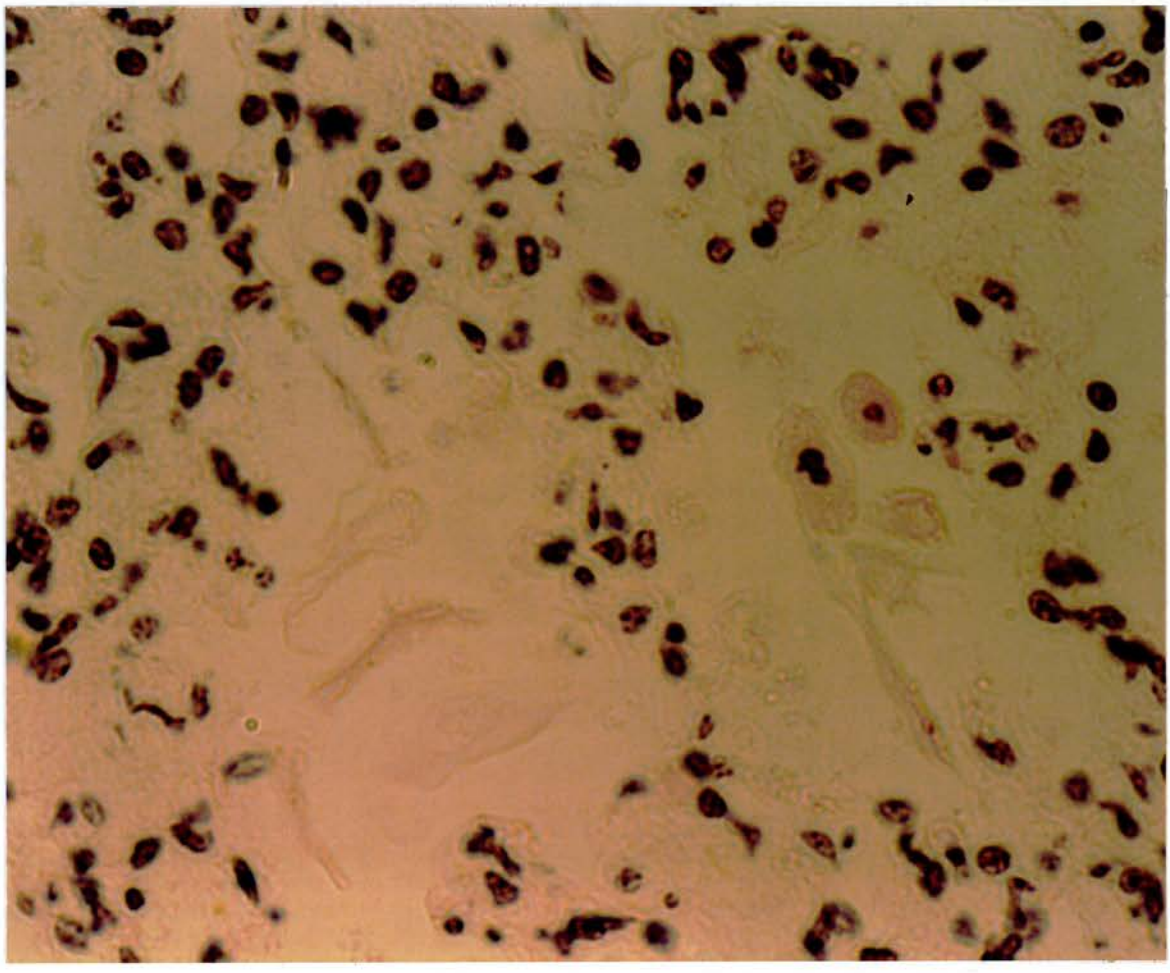

Fig 4-20 **Lung F2394; amniotic debris and nucleated epithelial cells in airways. Mononuclear inflammatory cells and oedema are evident. x864**

A summary of the histological features of lungs from all 65 cases is given in Table 26.

TABLE 26

Histopathological, serological and microbiological findings in 65 aborted fetuses with lesions present in lungs.

		Foetal (+) & maternal (x) serology					Description of lesions						
Foetus number	C/rp cm	IgG/ IgM	IBR	BVD	L.h'jo	Microbiology	mec/ sq	Oed	C	Fibr	Al mac	Int tissue MNI	PNI
F 017	82	-	-	-	-	C . pyogenes	+	-	-	-	+	-	+
F 028	68	-	-	x+	-	mixed cells	-	+	-	-	-	-	-
F 042	94	-	-	-	-	mixed cells	+	-	-	-	-	+	-
F 123	91	+	+	-	-	none	+	+	+	-	-	+	+
F 160	93	-	-	-	-	H.coli	+	-	+	-	+	-	+
F 239	90	-	-	-	-	mixed cells	+	-	+	-	-	+	-
F 468	60	+	-	-	-	N.H. coli	-	-	-	-	-	+	-
F 558	66	-	x	x	-	N.H. coli	+	-	-	-	-	+	-
F 785	63	-	x	-	+	C. pyogenes	+	-	-	+	+	-	+
F 797	50	-	-	-	-	N.H. coli	+	-	-	-	-	+	-
F 799	62	-	-	-	-	C. pyogenes	+	-	-	-	+	-	+
F 823	94	+	-	-	-	H. staph	+	-	-	+	+	+	+
F 886	70	-	-	-	x+	H. coli	+	-	-	-	-	+	-
F 888	65	-	+	-	-	H. strept	+	-	-	-	-	+	-
F 889	75	-	x	-	-	C. pyogenes	+	-	-	+	+	-	+
F 944	39	-	-	-	-	mixed cells	+	-	-	-	-	-	-
F 962	86	-	-	-	-	mixed cells	+	-	-	-	-	+	+
F 972	56	-	-	x	-	H. coli	+	-	-	-	-	+	+
F 983	86	-	-	x	-	H. coli	+	-	-	-	-	+	+
F 988	70	-	x	x	-	fungi	+	-	-	+	+	-	+
F 997	66	-	-	x	-	mixed cells	+	-	-	+	-	+	+
F 1011	66	+	+	-	-	mixed cells	-	-	+	+	+	-	+
F1032	76	+	+	-	-	mixed cells	-	-	+	+	+	-	+
F1039	83	+	-	-	-	B. lichen'fmis	-	+	+	-	-	-	-
F1065	86	-	-	x	-	mixed cells	+	-	-	+	-	+	-
F1148	56	-	-	x	-	mixed cells	+	-	-	+	-	+	+
F1219	49	-	x	-	-	mixed cells	-	-	-	+	+	-	-
F1221	87	-	x	-	-	mixed cells	+	-	-	+	-	+	+
F1224	80	-	-	x+	-	mixed cells	+	-	+	-	-	-	+
F1337	67	+	-	+	x	mixed cells	+	-	-	-	-	+	+
C1347	61	+	-	-	-	B.lichenf'mis	-	-	+	-	+	-	+
F1387	69	-	-	x	x	mixed cells	+	-	+	-	-	+	-

Key to abbreviations ;-

Mec/sq - Meconium or squamous cells
Oed - Oedema
C - Congestion
Fibr - Fibrosis
MNI- Mononuclear cell infiltration
PNI - Polymorphonuclear cell infiltration
Al mac - Alveolar macrophages

TABLE 26 (continued)

Foetus number	C/rp cm	Foetal (+) & maternal (x) serology: IgG/IgM	IBR	BVD	L.h'jo	Microbiology	Description of lesions: mec/sq	Oed	C	Fibr	Al mac	Int tissue: MNI	PNI
F1388	68	-	-	-	-	mixed cells	+	+	-	-	+	+	+
F1400	65	+	-	-	x	H coli	+	-	-	-	+	-	+
F1410	86	-	-	+	-	mixed cells	+	-	+	-	-	-	+
F1438	36	+	-	+	x	mixed cells	+	-	-	-	+	+	+
F1452	50	+	-	+	-	H.coli	-	-	+	-	-	+	+
F1462	78	-	-	x	-	mixed cells	-	-	+	-	-	-	+
F1492	90	-	-	-	-	mixed cells	+	-	+	-	-	-	-
F1505	68	+	-	x+	-	mixed cells	+	-	+	-	-	-	-
F1580	81	+	x	-	-	H.staphs	+	-	-	-	+	-	+
F1599	92	+	-	x+	-	mixed cells	-	-	+	-	-	+	+
F1600	56	-	-	-	x	N.H. coli	+	-	-	-	+	+	+
F1624	77	-	-	-	-	mixed cells	-	-	-	-	-	+	+
F1639	62	-	-	x	-	H.coli	-	-	+	-	-	-	+
F1720	46	+	-	-	-	B.lichenf'mis	-	-	-	-	-	+	+
F1795	75	-	-	-	-	C. pyogenes	+	-	+	+	+	-	+
F1981	56	+	-	-	-	fungi	+	-	+	-	+	-	+
F2000	84	-	-	-	-	mixed cells	-	-	-	-	-	+	+
F2394	56	-	-	-	-	mixed cells	+	-	+	+	-	-	-
F2589	55	+	-	-	-	fungi	+	-	-	-	+	-	+
F2591	76	+	-	x+	-	mixed cells	-	-	+	-	+	-	+
F2731	90	-	x	-	x	C. pyogenes	-	-	-	-	+	-	+
F2733	68	+	-	-	-	mixed cells	+	-	-	+	-	+	-
F2811	46	+	-	x+	+	C. pyogenes	-	-	+	-	-	+	+
F2879	79	-	-	-	-	fungi	+	-	+	-	+	-	+
F2917	80	-	-	x	-	none	-	-	-	-	+	+	+
F2984	74	+	-	x	x+	mixed cells	-	-	-	-	-	+	-
F3023	58	-	-	-	-	fungi	+	-	-	-	+	+	+
F3060	84	+	-	-	x	mixed cells	+	-	-	-	-	-	+
F3074	52	+	-	-	x+	none	+	-	-	-	-	+	+
F3078	46	-	-	-	-	mixed cells	+	-	-	-	-	+	+
F3223	88	+	-	-	x+	mixed cells	-	-	+	-	-	+	+

Additional findings

F 1358 - Vasculitis with thrombus formation in pulmonary blood vessels, with widespread polymorph cell infiltration.

F 1639 - Maternal blood titres to Brucella abortus at 1/80 rising to 3/320. Animal negative lesions at slaughter.

F 2000 - Foetal fluid titre of 1/128 to Toxoplasma gondii.

Key to abbreviations ;-

Mec/sq - Meconium or squamous cells
Oed - Oedema
C - Congestion
Fibr - Fibrosis
MNI- Mononuclear cell infiltration
PNI - Polymorphonuclear cell infiltration
Al mac - Alveolar macrophages

Of the 65 abortions with gross and microscopic lesions in the lungs, a possible infectious association was ascribed to 44. I.B.R infection was implicated in five cases as the single causal agent, and in a further six cases with a possible multiple aetiology involving L.hardjo (2 cases) and B.V.D (1 case). B.V.D was thought to be involved in 19 episodes and L.hardjo in 13, out of which four cases appeared to be affected by both conditions. Other bacterial or a fungal infection were implicated in the remainding cases.

Where no cause of abortion was found, the most common lesion of alveolitis with:

i) an interstitial mononuclear inflammatory cell infiltration (10/19 cases).

ii) a mixed inflammatory cell infiltration (8/19 cases).

iii) some fibrosis (3/19 cases).

Nine of the above cases presented concurrent microscopic placentitis in either cotyledon or placentome.

d) Lesions in fetal liver.

The histological structure of the bovine fetal liver differs from that seen in juvenile or adult cattle in the following respects:

i) the lobular pattern is rather indistinct.

ii) portal tracts are surrounded by wide zones of connective tissue which may contain immature leucocytes.

iii) parenchymal plates are irregular, the sinusoids are rather wide, and they contain numerous erythropoietic cells clustered together (Fig.4-21).

iv) as the fetus grows to term, the extent of extra-medullary haemopoiesis is reduced and few nucleated pro-erythroblasts are present.

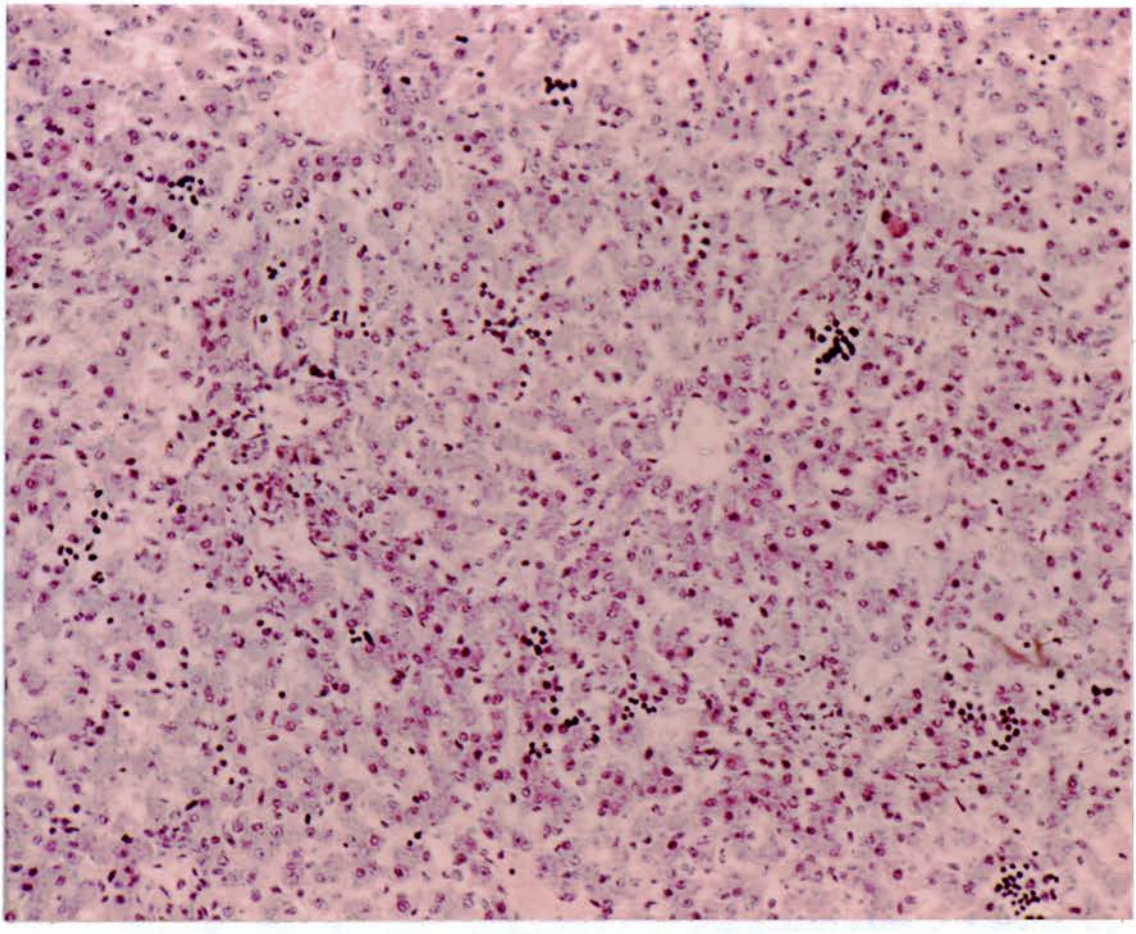

Fig. 4-21 **Liver at 190 days gestation; extensive extramedullary haematopoiesis within wide sinusoids. Connective tissue surrounding portal tracts contains immature leucocytes.x212**

Autolysis had occurred in the livers of 63 fetuses, and sections from these cases were discarded as being of limited diagnostic value. In passing, though, it is of interest to note that even when extensive degenerative changes had taken place in the liver parenchyma, erythropoietic cells were

still recognisable by their densely-staining nuclei with minimal karyorrhexis or pyknosis.

In 37 sections of fetal liver where autolytic changes were either minimal or absent, erythroblasts were commonly seen as small, polymorphic, densely-staining, basophilic clusters of cells sited within liver parenchyma. They were found in all livers from fetuses of crown-rump length 46cm or more, but their numbers decreased as gestational age increased. The gross lesion of hepatomegaly was associated with massive aggregations of erythroblasts in eight cases seen. In case F309, fetal hydrops was also present, together with inter-lobular oedema in the lungs as both a gross and microscopic lesion. Microscopically, a similar picture was found in livers from cases F028; F393; F558; F1337; F1387; F1388 (Fig.4-22); F1452; but there were additional lesions in fetal lungs of a mononuclear inflammatory cell infiltration, and a necrotic, haemorrhagic placentitis.

In case F1388 a coincidental placentitis was found, characterised by haemorrhage, and oedema. The amnion was also oedematous. Clinical pathology was unhelpful in establishing a direct cause of this abortion, which occurred against a herd background of acute L.hardjo infection, together with sero-positive evidence of concurrent B.V.D infection. Cases F558; F1337; F1387; F1452; all came from this same farm but gave positive titres of more than 1/400 to L.hardjo, and falling maternal titres or positive fetal sero-conversion to B.V.D.

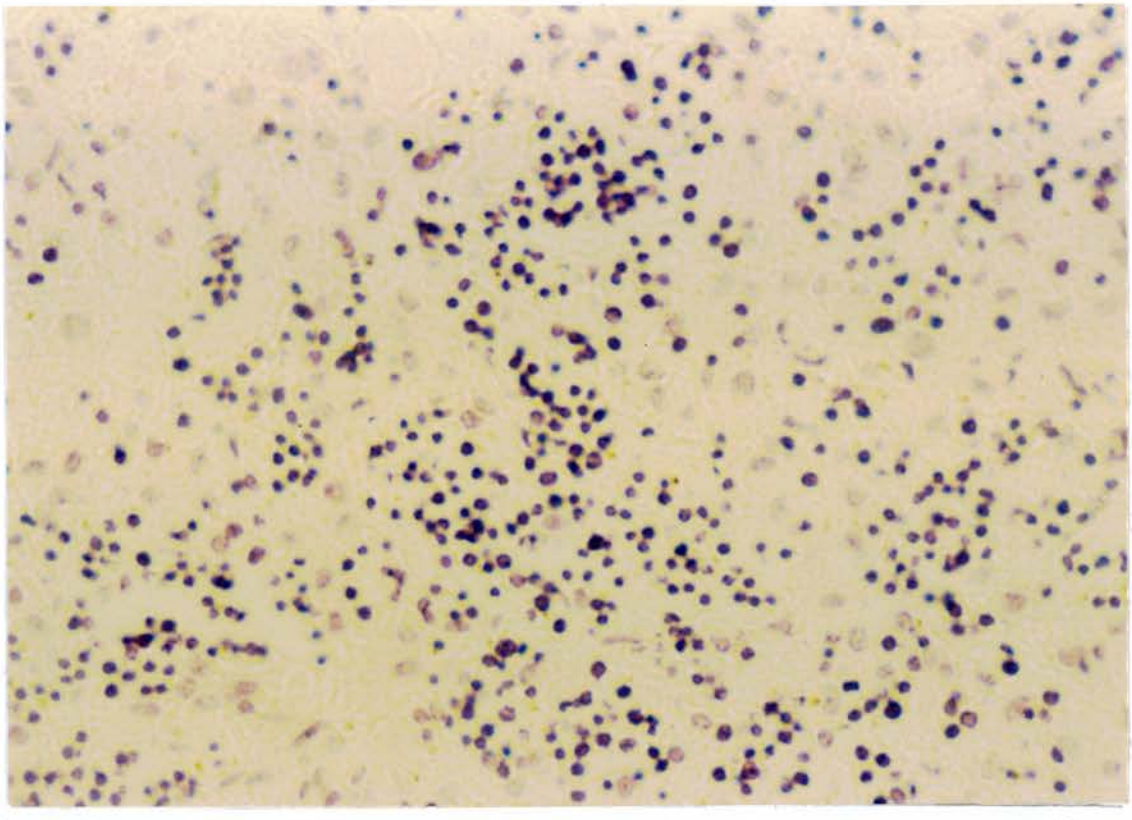

Fig. 4-22 **Liver F1385; markedly increased haemopioetic activity, still evident in autolysed liver which presented a gross lesion of hepatomegaly, x540**

The most frequently encountered lesions were peri-acinar leucocyte infiltrations (Fig.4-23) of varying intensity, and of congestion within the sinusoids. Occasional cases, eg.F1580 (Fig.4-24), presented focal necrosis with a mild inflammatory cell response. A summary of these lesions is set out in Table 27.

An infectious cause was suggested for 29/36 abortions where hepatic lesions were present. In 12 cases, there was serological evidence of B.V.D virus infection in either dam or fetus; the lesions consisted of peri-portal leucocyte infiltration. Focal hepatic necrosis was rarely seen (F1410; F2915) and occasional congestion was present (F1462; F1639) with erythrocytes packed into sinusoids. Six cases were

related to serological evidence of I.B.R infection in the dam, but only one case (F1580) disclosed lesions of focal hepatic necrosis.

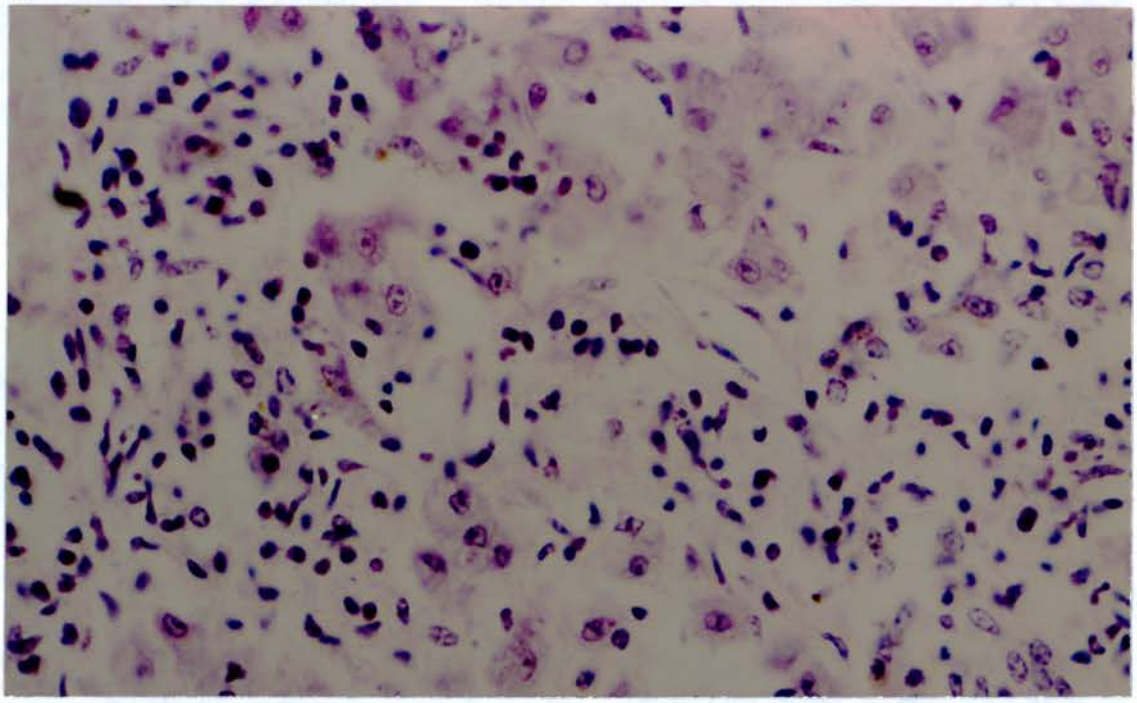

Fig. 4-23 Liver F2915; peri-acinar inflammatory cell infiltration and area of focal necrosis. Immature leucocytes are also present.x540

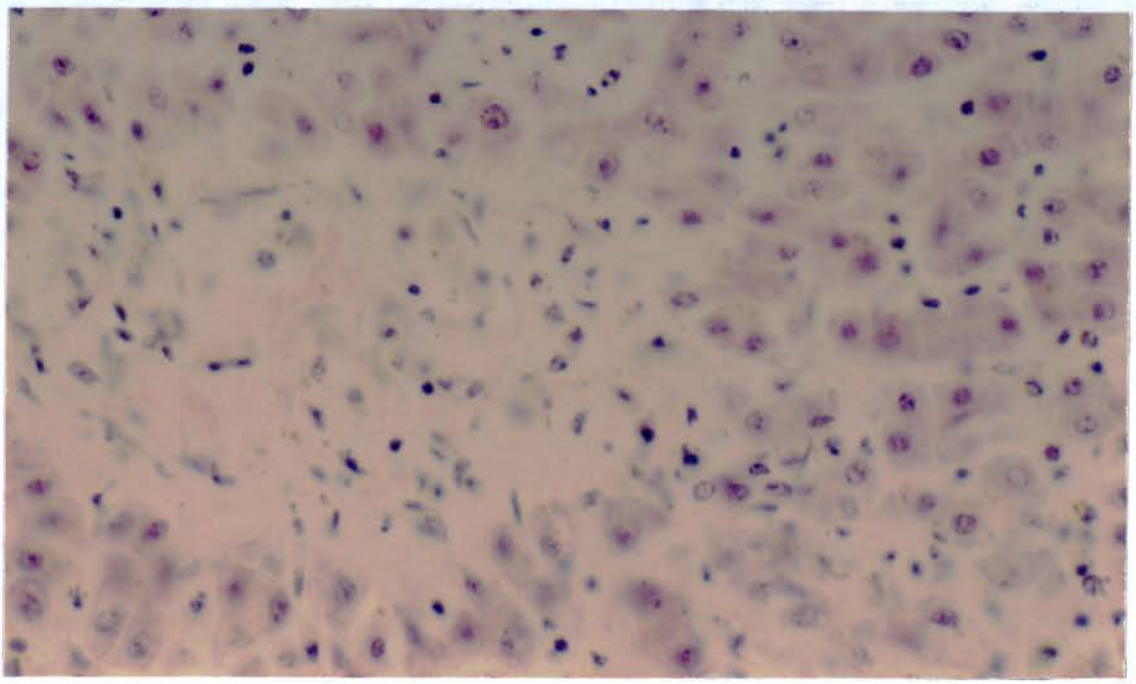

Fig. 4-24 Liver F1580; zonal centrilobular necrosis accompanied by a minimal inflammatory cell response. Extruded pyknotic nuclei are scattered throughout the lesion.x540

TABLE 27

Histological findings in livers of 37 aborted fetuses with related serological and microbiological results

		Fetal (+) & maternal (x) serology					Description of lesions			
Fetus No.	crown rmp cm	IgG IgM	IBR	BVD	L.hdjo	Micro biology	Cong	Coag	Leucs sinus	Peri-acnr leucs
F 017	82	-	-	-	-	C.pyogenes	-	-	-	+
F 123	91	+	+	-	-	mixed cells	-	+	+	-
F 309	78	-	-	-	-	H.coli	+	-	-	-
F 393	60	+	x	-	-	mixed cells	-	-	-	+
F 823	94	+	-	-	-	H.staph	-	+	+	-
F 962	86	-	-	-	-	mixed cells	-	-	-	+
F 983	86	-	-	-	-	H.coli	-	-	+	-
F1011	66	+	+	-	-	mixed cells	-	-	-	+
F1039	83	+	-	-	-	B.lichen'formis	-	-	-	+
F1224	80	-	-	+	x	mixed cells	+	-	-	+
F1251	94	-	-	-	-	B.lichen'formis	+	-	-	-
F1289	94	+	-	-	-	None	+	-	-	+
F1347	61	+	-	-	-	B.lichen'formis	+	-	-	+
F1358	79	-	x	-	-	H.coli	-	-	-	+
F1387	69	-	-	x	x	mixed cells	-	-	-	+
F1389	83	-	-	x	-	mixed cells	-	-	-	+
F1410	86	-	-	+	-	mixed cells	-	+	+	+
F1438	36	+	-	+	x	mixed cells	-	-	-	+
F1452	50	+	-	+	-	H.coli	-	-	-	+
F1462	78	-	-	x	-	mixed cells	+	-	-	+
F1556	80	+	x	x	-	H.coli	-	-	-	+
F1580	81	+	x	-	-	H.staph	+	+	+	+
F1624	77	-	-	-	-	mixed cells	+	-	-	+
F1639	62	-	-	x	-	H.coli	+	-	-	+
F1721	90	-	-	-	-	mixed cells	+	-	-	+
F1795	75	-	-	-	-	C.pyogenes	+	-	-	-
F1981	56	+	-	-	-	Fungi	+	-	-	+
F2000	84	-	-	-	-	mixed cells	+	-	+	+
F2394	56	-	-	-	-	mixed cells	+	-	-	-
F2526	64	-	-	-	-	S.typhimurium	-	-	+	+
F2591	76	+	-	x+	-	mixed cells	+	-	-	-
F2729	85	-	-	-	-	B.lichen'formis	+	-	-	-
F2733	68	+	-	-	-	mixed cells	+	-	-	-
F2811	46	+	-	x+	+	C.pyogenes	-	-	-	+
F2915	68	-	-	x	-	mixed cells	-	+	+	+
F3060	84	+	-	-	x	mixed cells	-	+	+	-
F3223	88	+	-	-	x+	mixed cells	+	-	-	+

Key to abbreviations :-

Cong - congestion
Coag - coagulative necrosis
Leucs. sinus - Leucocytes in sinusoids
Peri-acnr leucs - Peri-acinar leucocyte accumulation

The constancy of lesions from six cases associated with L.hardjo infection varied, from a moderate peri-portal aggregation of mixed inflammatory cells (F1387; F1389: F2811) to centri-lobular degeneration (F3060)

No diagnosis was made in five cases (F962; F1011; F1337; F1795; F2000), but coexisting lesions of conjunctival hyperplasia and placentitis were found elsewhere in these fetuses.

e)Lesions in other fetal tissues.

Adrenal gland.

Sections of adrenal gland were examined microscopically if a gross lesion was found, and these case numbers are listed: F017; F847; F1032; F1039; F1219; F1336; F1358; F1387; F1624; F3223.

Congestion was the most prominent feature, either in the zona fasciculata or zona reticularis, and in five cases (F847, Fig.4-25; F1032; F1039; F1219: F1387) a mild mixed leucocyte infiltration was perceptible. This lesion was not consistent with any particular diagnosis.

Kidney.

The vast majority of fetal kidneys were severely autolysed. Blocks from only seven cases were examined histologically - F823; F1011; F1032; F1981; F2000; F2394; F3223. Congestion was most common. Pale yellow-staining oxalate crystals were frequently located within the convoluted tubules of the cortex (Fig.4-26).

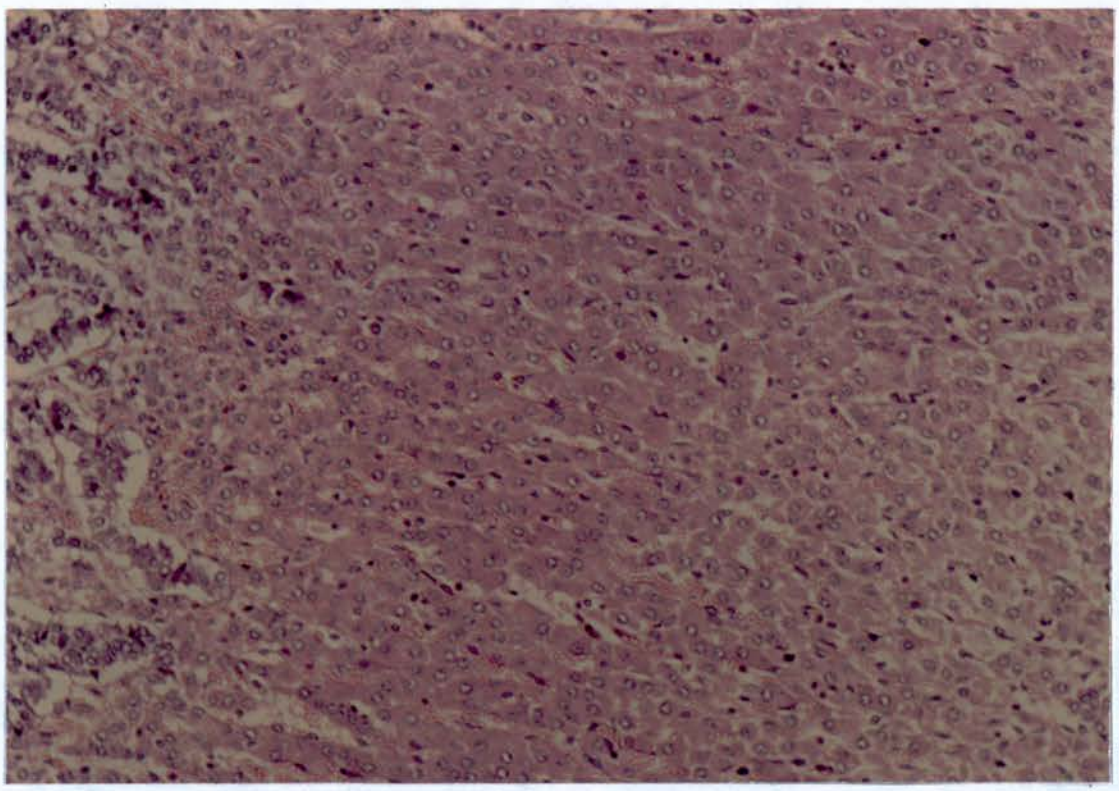

Fig 4-25 Adrenal F847; congestion of zona fasciculata with focal haemorrhage in zona glomerulosa. Sinusoids are grossly dilated and a few mononuclear inflammatory cells are present. x 212

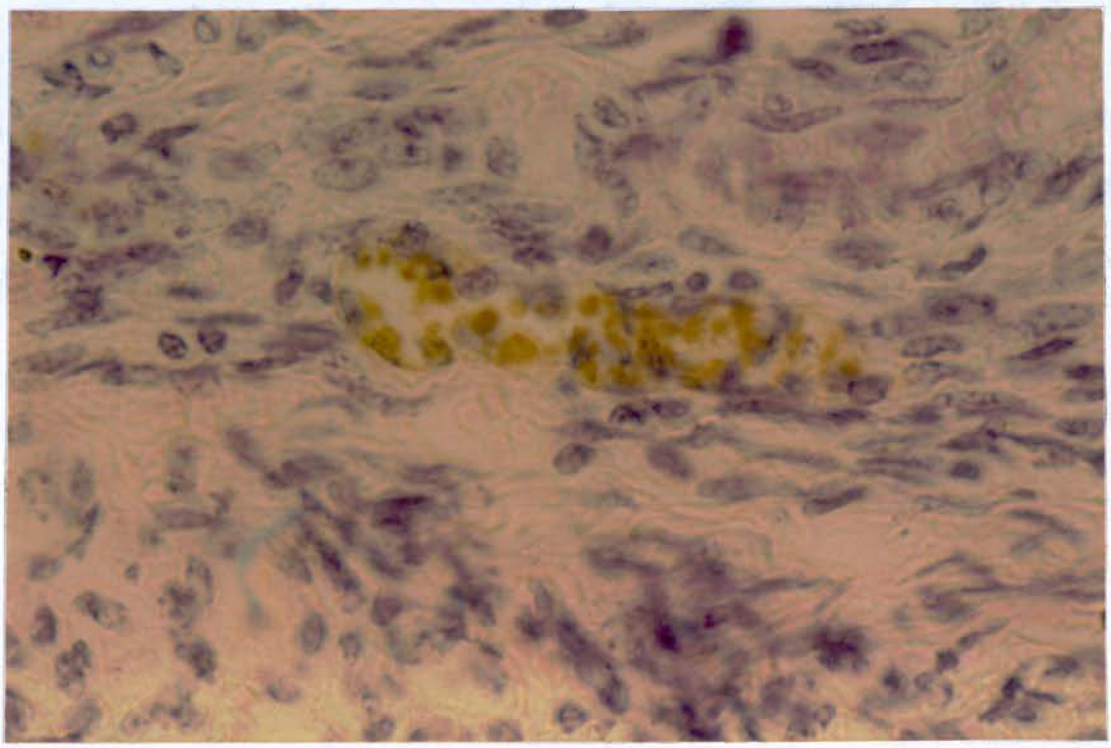

Fig 4-26 Kidney F1981; Yellow/brown staining oxalate crystals in a proximal tubule, accompanied by interstitial connective tissue proliferation. x 864

Thyroid gland.

Thyroid tissue was submitted for microscopic examination only if the gland appeared to be grossly hyperplastic. This was judged by the criteria of:

weight of fetus (kg) / 3 = thyroid weight (gm).

Only two thyroids were examined histologically (F017; F823). Haemorrhage and congestion in the peri-follicular connective tissue was found in F823. In case F017 the thyroid follicles appeared to be distended with colloid, which stained unevenly. No other evidence of hyperplasia was apparent.

4.5. Histopathological lesions in placental tissues.

a) Lesions in the placentome.

The placentome is mushroom-shaped. It is composed of a broad, short-stalked outgrowth from the stratum compactum of the endometrium and also of parts of the stratum spongiosum, with numerous parallel, vertically orientated, long and slender septa which extend from the maternal to the fetal side of the organ. The septa divide the organ into compartments or crypts, and horizontal or oblique branches project from these septa in parallel fashion.

The crypts are filled with profusely branching fetal villi which originate from the overlying chorio-allantoic membrane (C.A.M). The villi are composed of a connective tissue core in which run fetal blood vessels, covered by a layer of low cuboidal epithelium (Fig.4-27). This layer of trophoblast cells is composed of both mononuclear and binucleate cells, the numbers of the latter cells decreasing as pregnancy progresses.

The uterine epithelium is composed of cuboidal, mononuclear cells, but occasional binucleate giant cells can be seen in the fundic area of the crypts. A small number of mucus-secreting cells may be seen adjacent to the base of the fetal villi. This epithelium is supported by fibrocellular connective tissue, composed mostly of collagen fibres. Within this core of tissue runs a network of capillaries (Fig.4-28). Numerous lymphocytes may be in evidence.

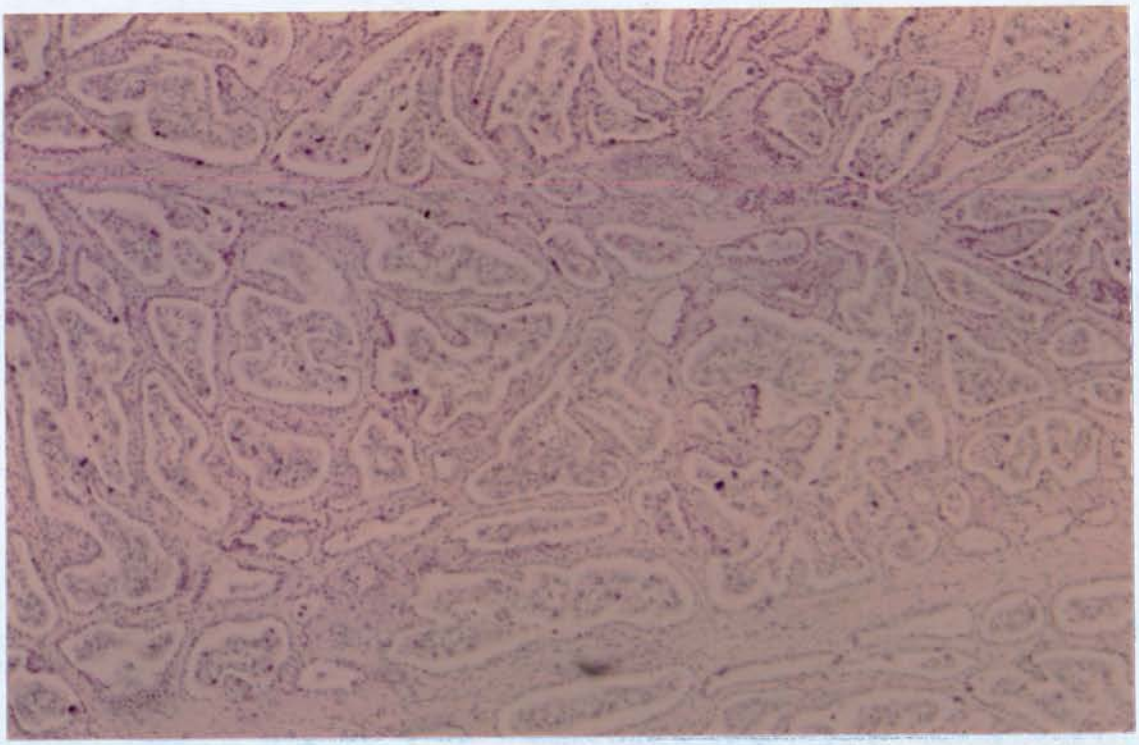

Fig. 4-27 Placentome from 190 day pregnancy. Branching maternal septa interdigitate with cores of fetal chorio-allantois. The separation between the tissues is artifact.x86

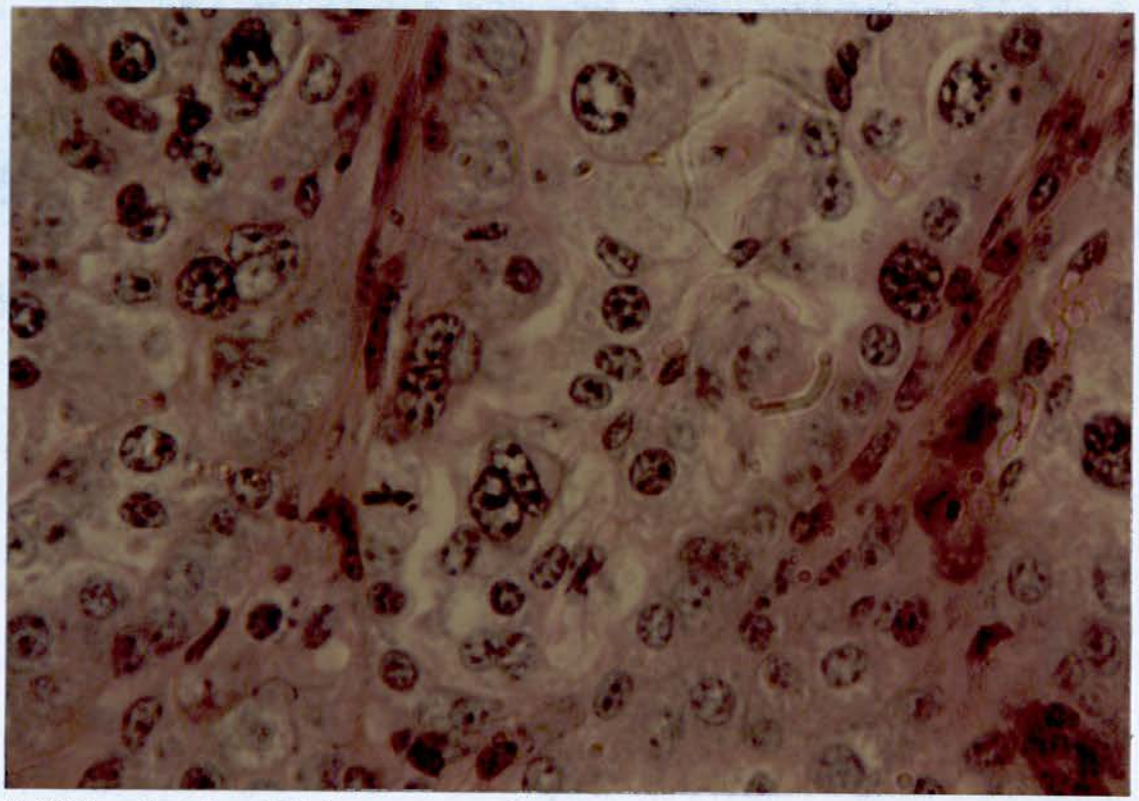

Fig. 4-28 Placentome from 240 day pregnancy. Maternal villi consisting of a cuboidal epithilium which encases a connective core and capillary network, in intimate contact and surrounding chorionic trophoblast where some binucleate cells are prominent.x864

A small portion of intercotyledonary chorio-allantois was often included in sections of placentome, attached to its outer border. Structurally, it was composed of two layers of cuboidal epithelium, separated by a broad layer of connective tissue stroma in which numerous blood vessels of varying size were found.

Sections of placentome were obtained from 40 aborted cattle and all presented lesions, the most common being variable degrees of degeneration and necrosis of chorionic villous epithelium. 32/40 cases presented evidence of a mild to moderate polymorphonuclear inflammatory cell infiltration within the stroma of the crypts, and 14 of these 32 cases produced an additional moderate mononuclear cell infiltration. Areas of focal necrosis, surrounded by an extensive zone of inflammatory cells, were found in 18/32 placentomes; they were accompanied by widespread haemorrhage and congestion in all but two cases.

Haemorrhagic lesions were noted in 20/40 placentomes, located mostly around the base of chorionic villi but extending down into the caruncular crypts. The lesions were characterised by widespread extravascular accumulations of erythrocytes and plasma within connective tissue, which occassionally leaked and localised between maternal and fetal epithelium at the feto-maternal interface. Blood vessels in the villous stroma were often packed with red blood cells and surrounded by areas of congestion. In the placentomes from F558 and F1358, these lesions were

accompanied by vasculitis in chorionic vessels; the additional finding of positive maternal or fetal titres to I.B.R infection was obtained from these cases. Serological evidence of infection with L.hardjo in aborted cows was found in a further six cases (F1337; F1339; F1387; F1388; F1600 -Fig.4-29; F3223). Thrombus formation with infarction, associated with fungal infection, led to zonal tissue necrosis and a massive inflammatory cell response in two cases (F1981; F3066).

Mineralisation of chorionic epithelial cells was found in 16/40 placentomes. In six cases, no diagnosis was made. In cases F962 and F1795, significant lesions co-existed in fetal eyelid but no diagnosis was made. Possible diagnoses that were made in cases presenting this lesion were B.V.D., I.B.R., and L.hardjo infections; in two cases a multiple aetiology was implicated.

Oedema and fibrosis of the stroma in the caruncular crypts were found in 14/40 sections, usually concurrent with other lesions; there were three cases where no other pathological changes were present, and no conclusions were drawn as to the cause of abortion.

The placentomes recovered from cases F1387; F1388; F1600; F1795; F2000; F2811; F2984 were notable, in that trophoblast sited near the base of many caruncular septa appeared to be degenerate; epithelial cells were ballooned, contained little stainable cytoplasm, and nuclei were in varying stages of karyorrhexis. Five of the cases were

associated with acute L.hardjo infection in the aborted dam: the remaining two (F1795; F2000) presented additional lesions of villitis and conjunctival hyperplasia.

Table 28 lists the cases where microscopic lesions were seen in placentome, and summarizes the observations made.

TABLE 28

Lesions described in 39 placentomes, together with related serological and microbiological findings.

		Fetal (+) & maternal (x) serology					Description of lesions						
							Chorion villi		Caruncular crypt				
Fetus No.	crown rmp cm	IgG IgM	IBR	BVD	L.hdjo	Microbiol	Min	Nec	PNI	MNI	F/O	Haem.	Vasc
F 017	92	-	-	+	-	mixed cells	+	+	-	+	-	-	-
F 042	94	-	-	-	-	c.pyogenes	+	+	+	+	+	-	-
F 558	66	-	x	x	-	N.H.coli	-	+	+	+	-	+	+
F 712	92	-	-	+	-	Mixed cells	+	+	-	+	-	-	-
F 785	63	-	x	-	+	C.pyogenes	-	+	+	-	-	+	-
F 799	62	-	-	-	-	mixed cells	-	+	+	-	+	-	-
F 886	70	-	-	-	x+	H.coli	+	+	+	+	-	-	-
F 889	75	-	x	-	-	C.pyogenes	-	+	+	+	-	-	-
F 961	36	-	-	x	-	mixed cells	-	+	+	+	-	-	-
F 988	70	-	x	x	-	fungi	-	+	+	--	-	+	-
F 962	86	-	-	-	-	mixed cells	+	+	+	+	-	-	-
F 988	70	-	x	x	-	fungi	-	+	+	+	-	+	-
F 997	66	-	-	x	-	C.pyogenes	+	+	+	+	-	+	-
F1011	66	+	+	-	-	mixed cells	+	+	+	+	-	-	-
F1032	76	+	x	-	-	mixed cells	-	+	+	-	+	+	-
F1144	--	-	-	-	-	none	+	+	+	+	+	-	+
F1224	80	-	-	x+	-	mixed cells	-	+	+	+	+	+	-
F1337	67	+	-	x	x	mixed cells	-	+	+	+	+	+	-
F1347	61	+	-	-	-	B.lich'formis	-	+	+	+	+	+	-
F1358	79	+	x	-	-	H.coli	-	+	+	-	+	+	+
F1386	88	-	-	-	-	mixed cells	+	+	+	+	-	+	-
F1387	69	-	-	x	x	mixed cells	+	+	+	-	-	+	-
F1388	68	-	-	-	-	mixed cells	-	+	+	+	+	+	-
F1400	65	+	-	-	x	H.coli	+	+	+	-	-	-	-
F1410	86	-	-	+	-	mixed cells	-	+	+	+	+	+	-

Key to abbreviations :-

Min :- Mineralisation
Nec :- Necrosis
Haem :- Haemorrhage
Vasc :- Vasculititis
PNI :- Polymorphonuclear cell infiltration
MNI :- Mononuclear cell infiltration
F/O :- Fibrosis and/ or oedma

TABLE 28 (continued)

Lesions described in 39 placentomes, together with related serological and microbiological findings.

		Fetal (+) & maternal (x) serology					Description of lesions						
							Chorion villi		Caruncular crypt				
Fetus No	crown rmp cm	IgG IgM	IBR	BVD	L.hdjo	Microbiol	Min	Nec	PNI	MNI	F/O	Haem	Vasc
F1600	56	-	-	-	x	mixed cells	-	+	+	+	-	+	-
F1624	77	-	-	-	-	H.coli	+	+	+	-	-	-	-
F1795	75	-	-	-	-	C.pyogenes	+	+	+	-	-	-	-
F1796	78	-	-	-	-	mixed cells	-	+	+	-	-	-	-
F1981	56	+	-	-	-	fungi	-	+	+	-	-	+	-
F2000	84	+	-	-	-	mixed cells	-	+	+	-	+	-	-
F2589	55	+	-	-	-	mixed cells	-	+	+	-	-	-	-
F2591	59	+	-	+	-	C.pyogenes	+	+	-	-	+	-	-
F2652	47	-	-	-	-	mixed cells	-	+	-	-	+	-	-
F2729	85	-	-	-	-	B.lich'formis	-	+	-	-	-	+	-
F2811	46	+	-	x+	+	C.pyogenes	+	+	+	+	-	-	-
F2984	74	+	-	x	x+	mixed cells	-	+	+	+	-	-	-
F3045	42	-	-	-	-	C.pyogenes	-	+	+	-	-	+	-
F3049	71	-	-	-	-	mixed cells	+	+	+	-	+	+	-
F3060	84	+	-	-	-	fungi	-	+	+	-	-	+	-
F3223	88	+	-	-	x+	mixed cells	-	+	+	-	-	+	-

Key to abbreviations :-

Min :- Mineralisation
Nec :- Necrosis
Haem :- Haemorrhage
Vasc :- Vasculititis

PNI :- Polymorphonuclear cell infiltration
MNI :- Mononuclear cell infiltration
F/O :- Fibrosis and/ or oedma

b) Lesions in cotyledon and chorio-allantoic membranes.

Extensive epithelial desquamation, degeneration, and necrosis were obvious features in 51/54 tissue sections examined. Karyorrhexis or pyknotic changes were seen in both mononuclear and binucleate cells. These changes were accompanied by a generalised leucocyte infiltration of the villous stroma in 38/51 cases, but a significantly more intense focal inflammatory response surrounded areas of necrosis in bacterial (e.g. F017; F988 Fig.4-30; F2811), and fungal (e.g. F1981; F3023) placentitis. In six cases with of severe focal villitis where an infectious aetiology could not be related to the abortions, conjunctivitis was also encountered and in two cases (F962; F1462, Fig. 4-31) an amnionitis was noticed.

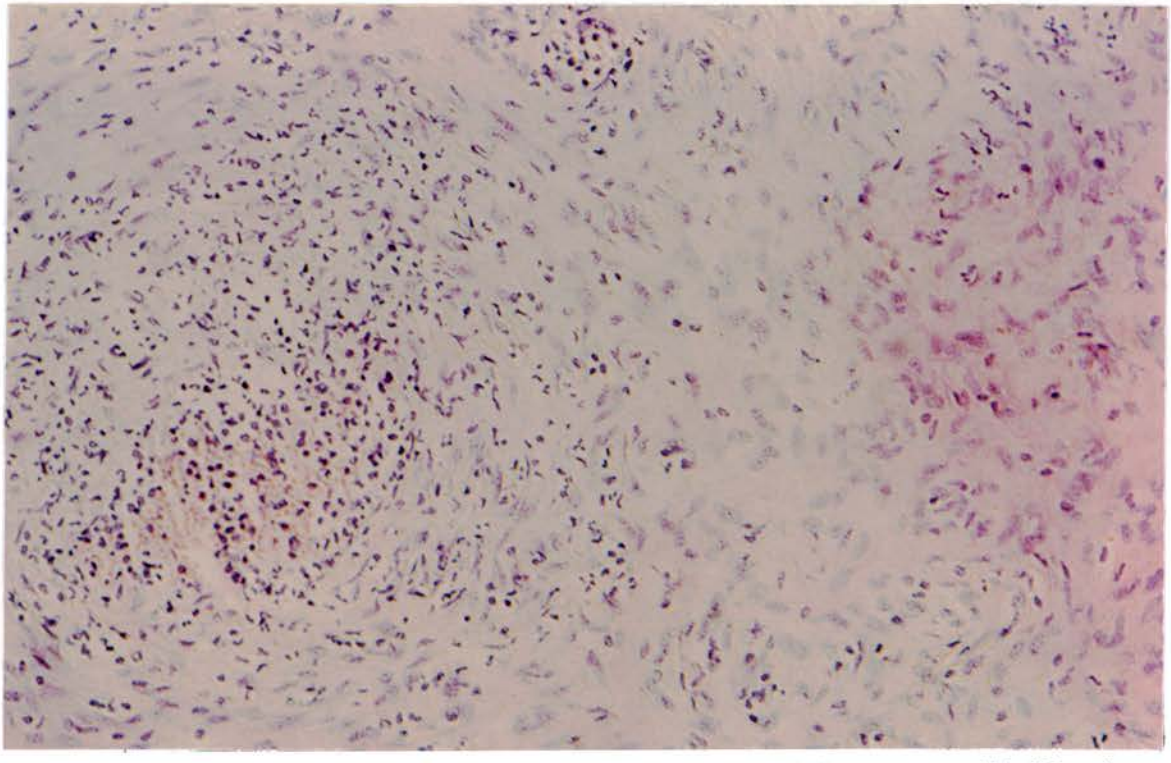

Fig. 4-29 **Placentome F1603 haemorrhagic placentitis with focal inflammatory cell infiltration, surrounded by areas of maternal villous congestion and oedema. x 212**

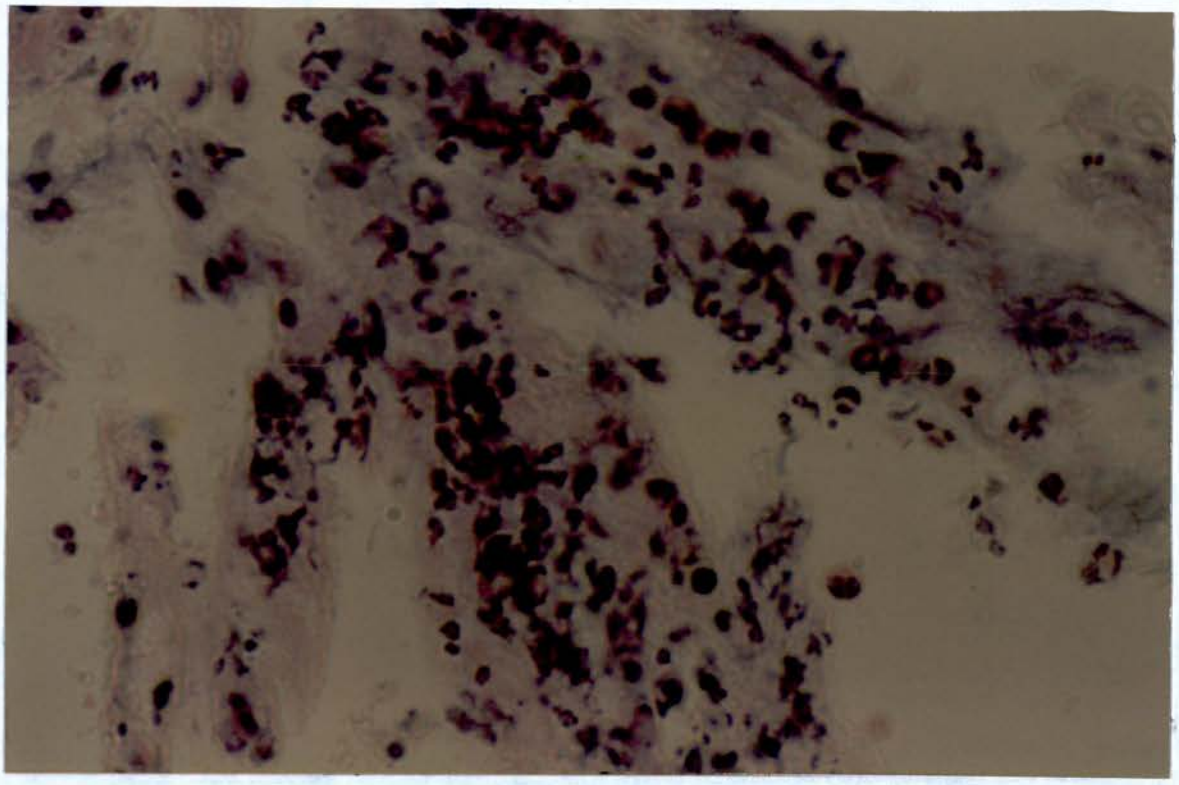

Fig. 4-30 Cotyledon F988; polymorphonuclear inflammatory cell infiltration and fetal villous necrosis associated with haemolytic Staphylococcal infection. x 864

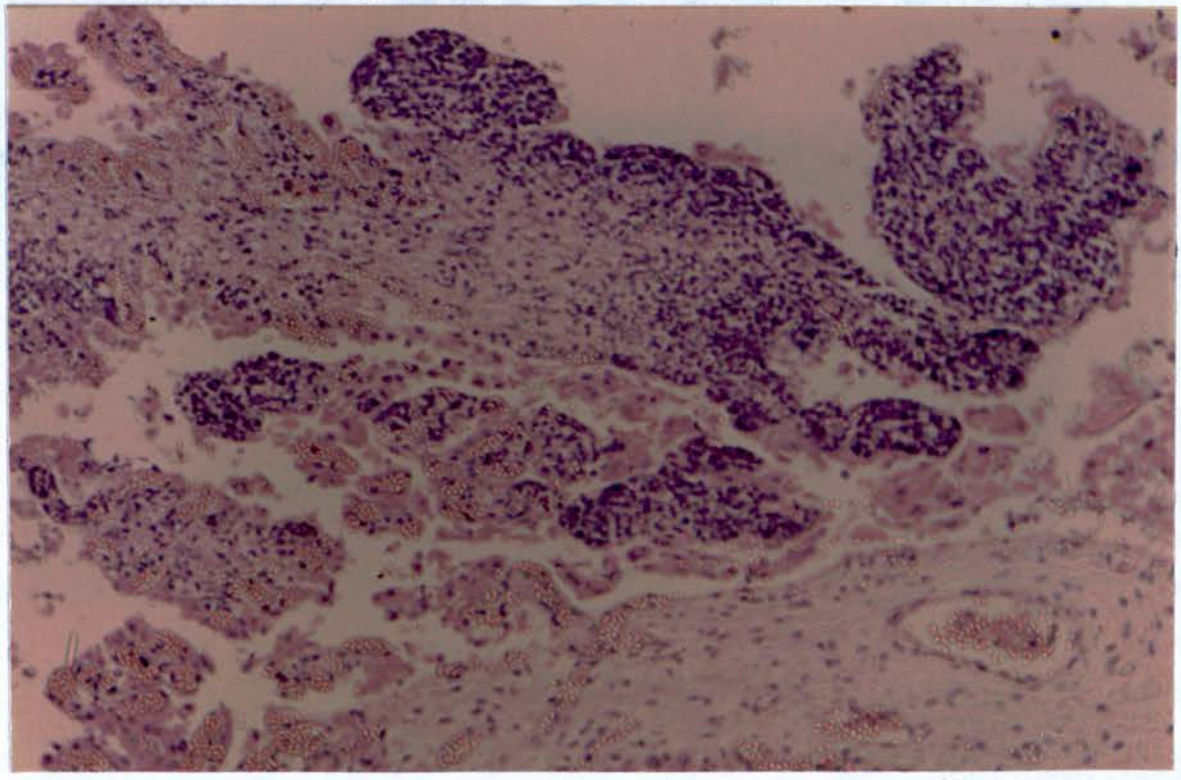

Fig. 4-31 Cotyledon F1462; a heavy mixed cell inflammatory cell infiltrate, predominately neutrophils, in necrotic chorio-allantoic villi, x212.

Mononuclear inflammatory cell infiltrations were found in villous connective tissue of 18/54 sections; the cells were mainly lymphocytes with some plasma cells present. Serological evidence of B.V.D virus involvement was associated with 6/18 cases, but no conclusions were reached with a further 7/18.

Oedema was a feature in 24/54 sections that were studied. It affected the connective tissue stroma, often with an accompnying hyperaemia in villous capillaries or larger blood vessels. F1981 had the additional lesion of widespread vasculitis (Fig.4-32). A concurrent placentitis was present in six of these cases.

Mineralisation was evident in 18/54 sections of C.A.M, of which only half had a diagnosis attributed to them. Fragments of degenerating trophoblast were seen seperated from the chorionic vill, which stained heavily with haematoxylin. In 10/18 of these cases a necrotic placentitis with leucocyte infiltration was present; six cases also presented the lesion of conjunctival hyperplasia, including two (F962; F1462) with a concurrant amnionitis.

In five cases (F1387; F1388; F1600; F2811; F2984) the trophoblast at the tips of chorionic villi were under-going degenerative changes, typified by extensive vacuolation, ballooning, absence of cytoplasm from cells, and karyorrhexis. This lesion was related to high antibody titres - 1/1600 to 1/2560 - in maternal serum samples to L.hardjo, indicative of recent infection.

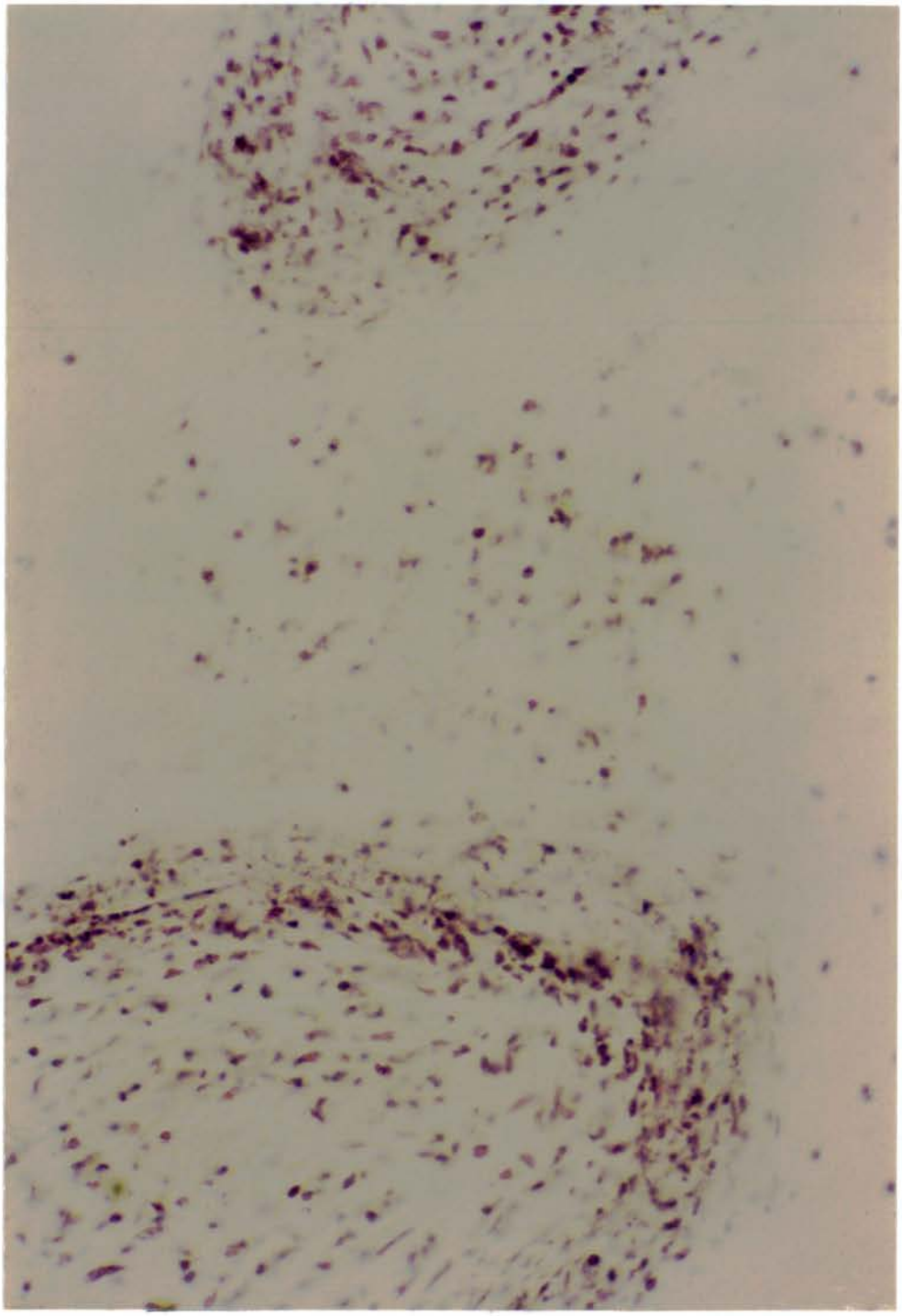

Fig. 4-32 Chorio-allantois F1981; Grade 11 chorionitis. Neutrophil infiltration of chorionic blood vessel wall, extending throughout the chorion. x540

Table 29 records the major lesions encountered in 55 tissue sections of C.A.M.

TABLE 29

A summary of lesions in cotyledon and C.A.M. in 55 abortion cases, together with related serological and microbiological findings

		Fetal (+) & maternal (x) serology					Description of lesions					
Fetus No.	crown rmp cm	IgG IgM	IBR	BVD	L. hdjo	Microbiol	Nec	Oed	Min	Bact T'blast	MNI	PNI
F 017	82	-	-	-	-	C.pyogenes	+	-	-	+	-	+
F 042	94	-	-	-	-	mixed cells	+	-	+	-	-	+
F 125	32	-	-	-	-	mixed cells	-	-	+	-	+	-
F 160	93	-	-	-	-	H.coli	+	+	-	+	-	+
F 240	70	-	-	-	-	mixed cells	-	+	-	-	-	-
F 586	21	-	-	-	-	mixed cells	+	+	-	-	+	+
F 632	59	-	-	-	-	C.pyogenes	+	-	-	-	-	+
F 712	92	+	-	+	-	none	+	-	-	-	-	-
F 789	76	+	-	-	-	mixed cells	+	-	-	-	-	+
F 797	47	-	-	-	-	N.H.coli	+	-	+	-	+	-
F 799	62	-	-	-	-	mixed cells	+	+	-	-	-	+
F 886	70	-	-	-	x+	N.H.coli	+	-	-	-	+	+
F 888	65	-	-	-	-	H.strept	+	-	-	+	-	+
F 889	75	-	x	-	-	C.pyogenes	+	+	-	+	+	+
F 961	32	-	-	x	-	mixed cells	+	-	-	-	+	-
F 962	86	-	-	-	-	mixed cells	+	+	+	-	-	+
f 972	56	-	-	x	-	N.H.strept	+	-	+	-	+	-
F 983	86	-	-	x	-	H.coli	+	-	+	-	+	-
F 988	70	-	x	x	-	mixed cells	+	-	-	-	-	-
F 997	66	-	-	x	-	C.pyogenes	+	+	-	-	-	+
F1011	66	+	-	-	-	mixed cells	+	+	-	-	-	+
F1032	76	+	x	-	-	C.pyogenes	+	+	-	-	-	+
F1040	85	+	-	-	-	mixed cells	+	-	-	-	-	+
F1056	40	-	-	-	-	H.coli	+	-	+	-	-	+
F1103	59	-	x	x	-	H.coli	+	-	-	-	-	+
F1144	--	-	x	-	-	mixed cells	+	+	+	+	-	+
F1148	56	-	-	x	-	N.H.staph	+	-	+	-	-	-
F1221	87	-	x	-	-	mixed cells	+	-	-	-	-	-
F1224	80	-	-	x+	-	mixed cells	+	+	+	-	-	+
F1336	94	+	-	-	-	none	+	+	-	-	-	-
F1347	61	+	-	-	-	B.lich'formis	+	+	+	-	-	+
F1358	79	+	x	-	-	H.coli	-	-	+	-	+	+
F1386	88	-	-	-	-	mixed cells	+	-	+	-	-	-

key to abbreviations;-

Nec - Necrosis
Oed - Oedema
Min - Mineralisation
Bact. T' blast - bacteria in trophoblast
MNI - Mononuclear cell infiltration
PNI - Polymorphonuclear cell infiltration

TABLE 29 (continued)

A summary of lesions in cotyledon and C.A.M. in 55 abortion cases, together with related serological and microbiological findings

		Fetal (+) & maternal (x) serology					Description of lesions					
Fetus No.	crown rmp cm	IgG IgM	IBR	BVD	L. hdjo	Microbiol	Nec	Oed	Min	Bact T'blast	MNI	PNI
F1410	86	-	-	+	-	mixed cells	+	+	+	-	-	+
F1462	78	-	-	x	-	mixed cells	+	+	+	-	-	+
F1600	56	-	-	-	x	mixed cells	+	-	-	-	-	-
F1624	77	-	-	-	-	mixed cells	+	-	+	-	-	+
F1720	46	+	-	-	-	none	+	+	-	-	-	+
F1795	75	-	-	-	-	C.pyogenes	+	+	-	-	-	+
F1981	56	+	-	-	-	fungi	+	+	-	-	-	+
F2351	60	+	-	-	-	none	+	-	+	-	-	-
F2589	55	+	-	-	-	fungi	+	-	-	-	-	+
F2652	47	-	-	-	-	mixed cells	+	-	-	-	-	+
F2729	85	-	-	-	-	B.lichen'formis	+	-	-	-	-	+
F2733	68	+	-	-	-	mixed cells	+	-	-	-	-	+
F2811	46	+	-	x+	+	C.pyogenes	+	-	-	+	+	+
F2915	68	-	-	x	-	H.coli	+	-	-	-	+	+
F2917	80	-	-	x	-	none	+	-	-	-	+	+
F2984	74	+	-	x	x+	mixed cells	+	-	-	-	+	-
F3023	58	-	-	-	-	fungi	+	+	+	-	-	+
F3045	33	-	-	-	-	C.pyogenes	+	+	-	-	-	-
F3049	52	-	-	-	-	mixed cells	-	+	-	-	+	+
F3060	84	+	-	-	x	mixed cells	+	-	-	-	-	+
F3074	52	+	-	-	x+	H.coli	+	+	-	-	+	-
F3078	46	-	-	-	-	none	+	+	-	-	+	-

key to abbreviations:-

Nec. - Necrosis
Oed. - Oedema
Min. - Mineralisation
Bact T' blast - bacteria in trophoblast cells
MNI - Mononuclear cell infiltration
PNI - Polymorphonuclear cell infiltration

Lesions of the amnion.

Amnion was retrieved for examination in ten cases only, when veterinary assistance was required to deliver an aborted fetus. It was difficult to obtain fresh amnion from abortion cases in which a retained placenta was the only clinical material available from the cow. The lesions observed most frequently were of oedema and fibrosis, together with focal aggregations of mixed inflammatory cells within the supporting connective tissue. In 3/10 sections, a distinct amnionitis was present, characterised by epithelial necrosis, mineralisation, fibrosis, and a predominantly mononuclear inflammatory cell infiltration (Figs.4-33).

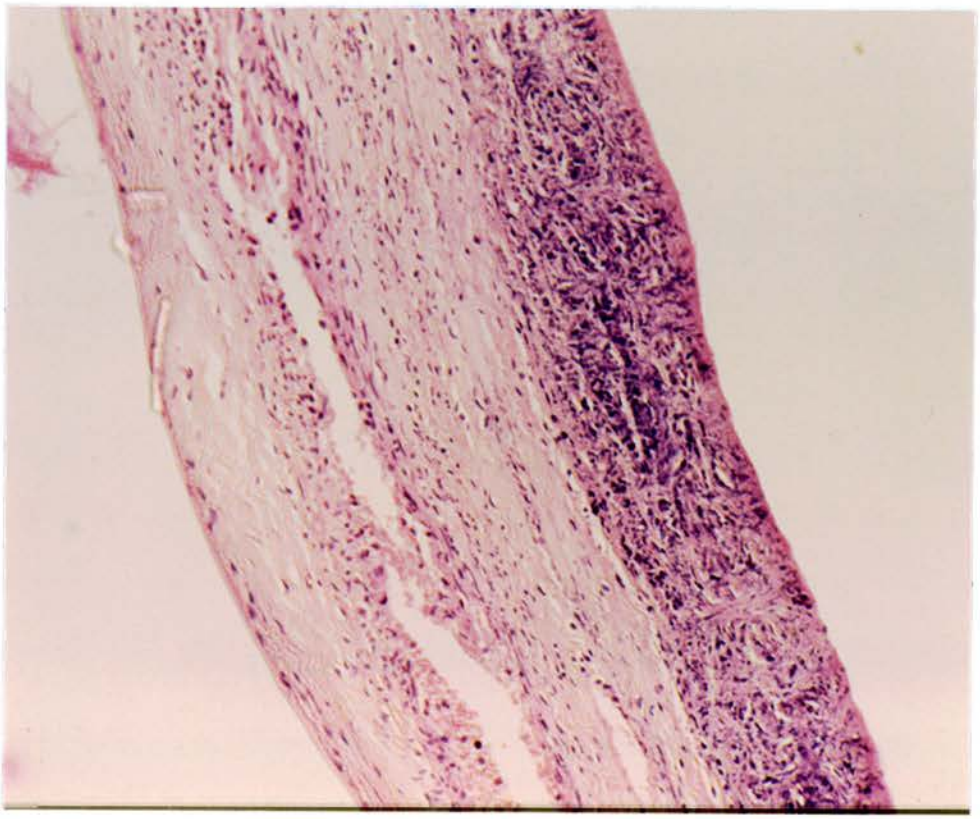

Fig. 4-33 **Amnoin F 847; severe amnionitis. Marked fibrosis, mineralisation, and heavy inflammatory cell infiltration which is predominately mononuclear cells, with areas of focal necrosis. x212**

Epithelial hyperplasia was a feature of three sections examined with an absence of other lesions. These amniotic plaques were composed of stratified epithelium which appeared to be cornified. The surface cells were larger than those normally found in the epithelial monolayer.

The lesions are recorded in Table 30.

TABLE 30

Lesions in the amnion from 10 abortion cases with related serological and microbiological findings

		Fetal (+) & maternal (x) serology					Description of lesions					
Fetus No.	crown rmp cm	IgG IgM	IBR	BVD	L.hdjo	Micro biology	Nec	Oed	Fib	Min	Epith.	Leuc infl
F 797	50	-	-	-	-	N.H.coli	-	+	-	-	-	+
F 962	86	-	-	-	-	mixed cells	+	-	+	+	-	+
F1040	85	+	-	-	-	mixed cells	+	+	+	+		+
F1065	88	-	-	x	-	mixed cells	-	-	-	-	+	-
F1388	68	-	-	-	-	mixed cells	-	+	+	-	-	-
F1400	65	+	-	-	x	H.coli	-	+	-	-	-	-
F1462	78	-	-	x	-	mixed cells	-	+	+	+	-	+
F2591	76	+	-	+	-	C.pyogenes	+	-	+	-	-	+
F2729	85	-	-	-	-	B.lich'formis	-	+	+	-	+	-
F3233	88	+	-	-	x+	mixed cells	-	-	-	-	+	-

Key to abbreviations;-

Nec - Necrosis
Oed - Oedema
Fib - Fibrosis
Min - Mineralisation
Epith - Epithelialisation
Leuc infl - Leucocyte infiltration

4.6. Histopathological lesions from abortions associated with an infectious aetiology.

a) Lesions associated with bacterial (except L.hardjo) and fungal infections.

Of the tissues examined, those of placenta and lung produced the most consistent lesions (Fig.4-34). Necrotic placentitis was a feature of 9/10 cases, with an accompanying polymorph inflammatory cell infiltration and a non-specific sub-conjunctival inflammatory reaction. Lesions of broncho-pneumonia were specific in these cases; the airways were densely packed with exudate and inflammatory cells, usually accompanied by a mixed inflammatory cell infiltration of interstitial tissues. C.pyogenes was isolated from lungs with this pathological picture in four cases as the sole infective agent identified.

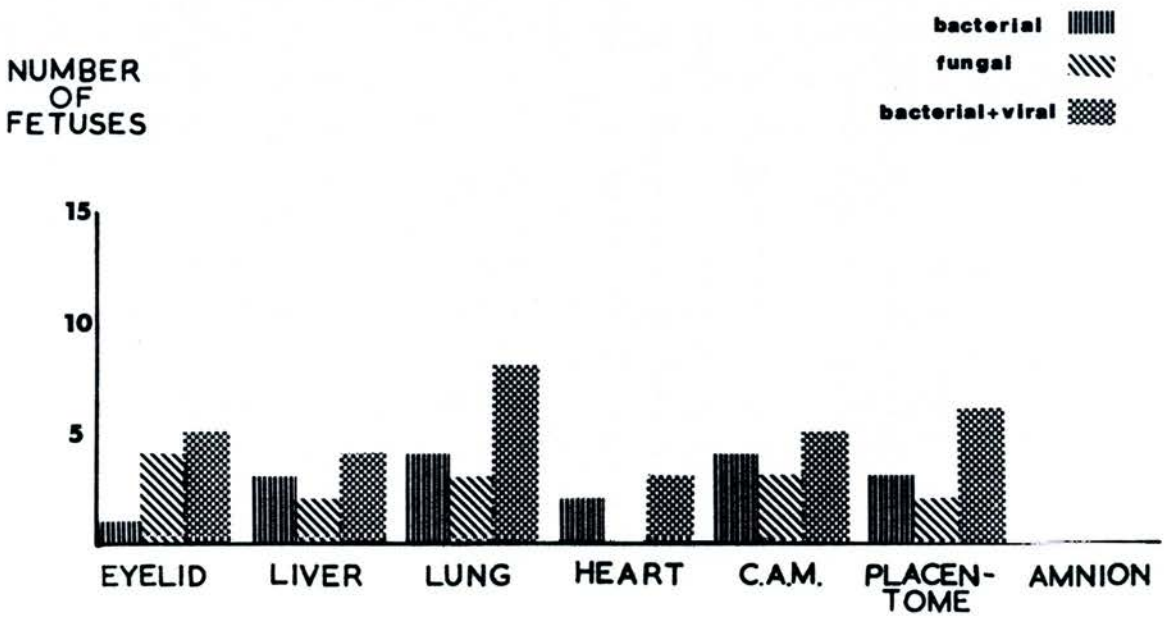

Fig 4-34 **Distribution of histopathological lesions found in tissues of 20 fetuses with a diagnosis of either bacterial or fungal infectious abortion.**

The small number of fungal infections encountered produced lesions in the eyelid of folliculitis and conjunctivitis, related to hyphal elements adjacent to the mucocutaneous junction. In these cases, the accompanying placental lesion was of severe congestion, haemorrhage, with massive zonal necrosis in placentome and a dense inflammatory cell reaction.

In five cases bacteria were found in degenerating trophoblast cells, and in one case within a focal abscess located in the caruncle (F983.Fig.4-35).

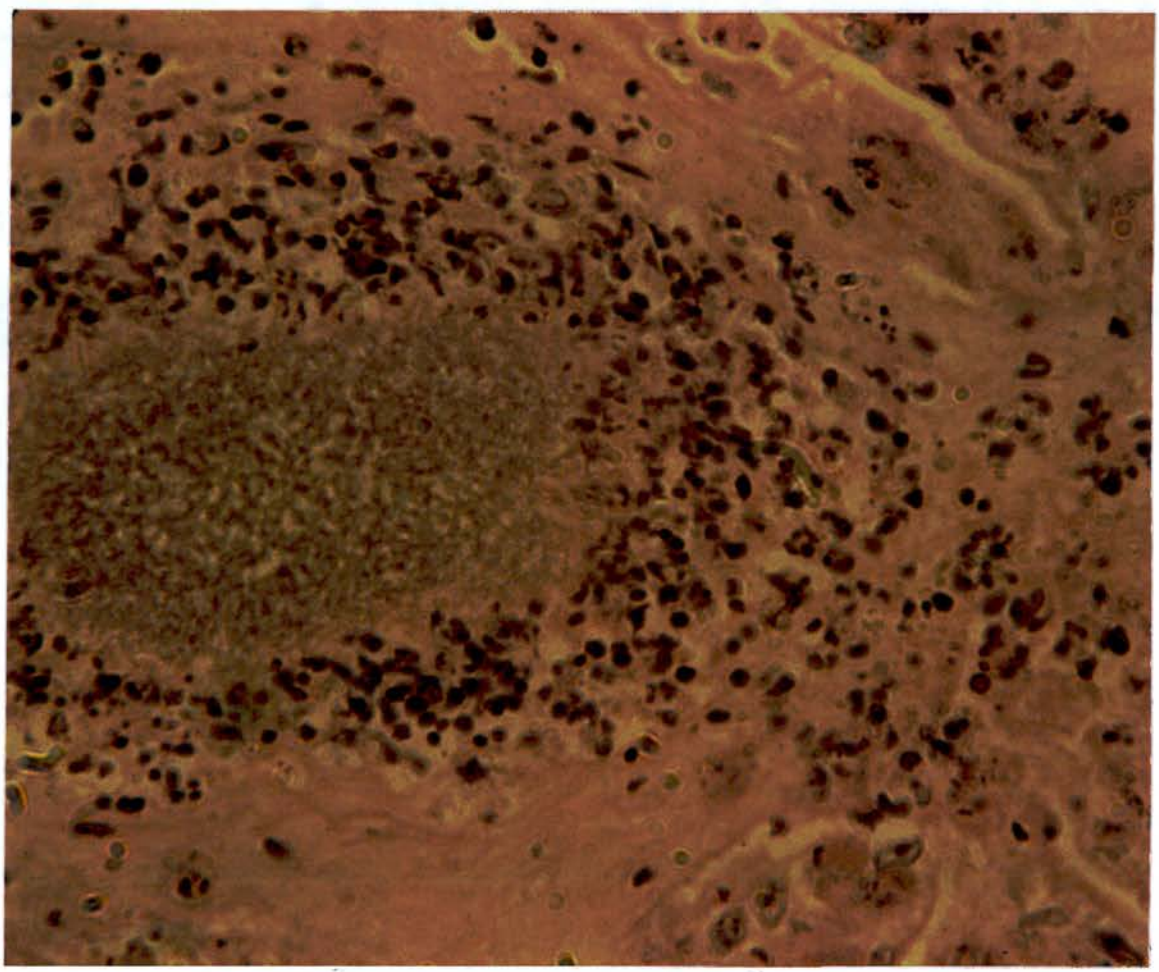

Fig 4-35 **Caruncle F983; necrotic debris and bacteria surrounded by a mixed but predominately polymorph inflammatory cell infiltration and fibroblasts.x540**

When a peracute maternal septicaemia was directly related to an abortion, e.g. S.typhimurium, pathological changes in both fetal and placental tissues were minimal. A similar picture was found when serological evidence implicated I.B.R infection but pure isolates of bacteria such as haemolytic Staphylococci were recovered from aborted fetal tissues at the same time.

b) Lesions associated with bacterial infections (excluding L.hardjo) and serological evidence of pre-existing viral infection.

Of the ten cases in this category, a necrotic placentitis was seen with a more diffuse inflammatory cell infiltration, coupled with varying degrees of oedema. In particular, mononuclear cells and fibrocytes infiltrated the fibrous tissue of caruncular crypts; in some cases the reaction could be intense. Where I.B.R was implicated in the aetiology, vasculitis and oedema were evident although the degree of necrosis in placental tissues could be almost minimal.

Fetal lesions were diverse. Evidence that fetal bacterial infection had occurred via placental spread was still present since lesions of broncho-pneumonia were seen in 8/10 cases, but were also accompanied by lesions of a moderate interstitial mononuclear inflammatory cell reaction. 5/10 cases had a related sub-conjunctival inflammation. In these same cases, other organs examined presented lesions of a generalised mixed leucocyte infiltration, which

may suggest a possible haematogenous route for any viral componant contributing to the death of the fetus.

c) Lesions associated with L.hardjo infection.

Few lesions in eyelid or heart were associated with fetal infection by this organism, as shown in Fig.4-36.

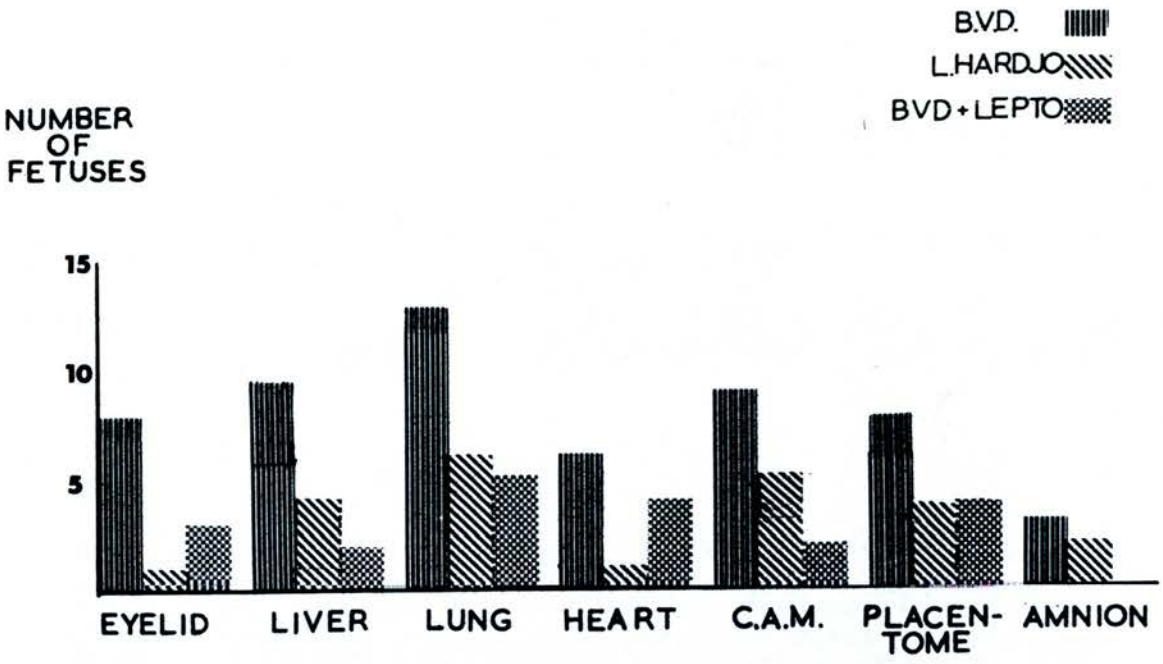

Fig 4-36 Distribution of histopathological lesions found in tissues of 37 fetuses associated with a diagnosis of Bovine Viral Diarrhoea virus or L. hardjo infection, either in single or multiple infections

Although placental lesions of haemorrhagic placentitis were found in seven cases examined, characterised by congestion, oedema, and frank haemorrhage with extensive focal villous degen eration, necrosis and a variable mixed inflammatory cell infiltration, hypoxic change was the most notable feature.

The lung lesions in 4/7 cases were of a mixed inflammatory cell infiltration of interstitial tissues

together with accompanying congestion and haemorrhage. A bronchopneumonia was found in only one case.

Erythroblastosis was a feature in five cases.

Should serological evidence of B.V.D infection also be present, as in five abortions investigated, the pattern of lesions did not vary save for a mild, diffuse mononuclear inflammatory cell infiltration in eyelid and other soft tissues.

d) Lesions associated with a sero-positive result for B.V.D virus infection in either fetus or dam.

Serological evidence pointed to B.V.D virus as being the single most common infection associated with episodes of abortion which were investigated; 26 cases were so labelled. In a further three cases, evidence of a secondary bacterial infection was found.

Villous necrosis was a common feature of placental tissues examined, together with mineralisation, fibrosis, and oedema which were seen in over half the sections.

A generalised infiltration of fetal soft tissue organs by mononuclear inflammatory cells was the non-specific lesion recorded (Fig.4-37), with the occasional finding of focal myocardial degeneration or necrosis in 6/18 cases (see Table 25). Interstitial congestion with a mononuclear inflammatory cell infiltration and some fibrosis was observed in the lungs of these same cases.

e) Lesions associated with a sero-positive result in either fetus or dam for I.B.R infection.

I.B.R was the only aetiological agent identified in 7/9 cases that were serologically positive for this infection (Fig.4-37).

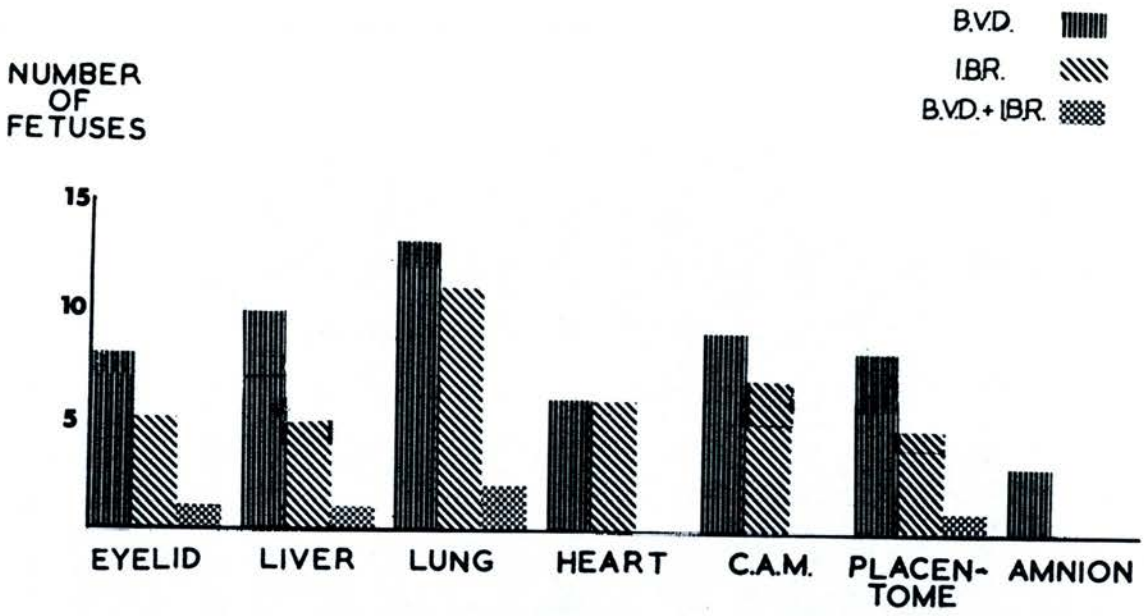

Fig 4-37 Distribution of histopathological lesions found in tissue of 40 fetuses associated with a diagnosis of Bovine Viral Diarrhoea virus or Infectious Bovine Rhino-trachitis virus, either in single or multiple infections

Placental lesions were essentially those of necrosis, congestion, and a variable mixed leucocyte infiltration which could be reduced to almost negligable proportions in some cases. Occasional haemorrhage and vasculitis were located in inter-cotyledonary connective tissue stroma and at the base of maternal caruncles.

Lung lesions included congestion and a mixed inflammatory cell infiltration of the parenchyma, with variable

degrees of haemorrhage. Neither vascular lesions nor areas of focal necrosis were found. Focal myocarditis was the major lesion in the heart (Fig.4-38).

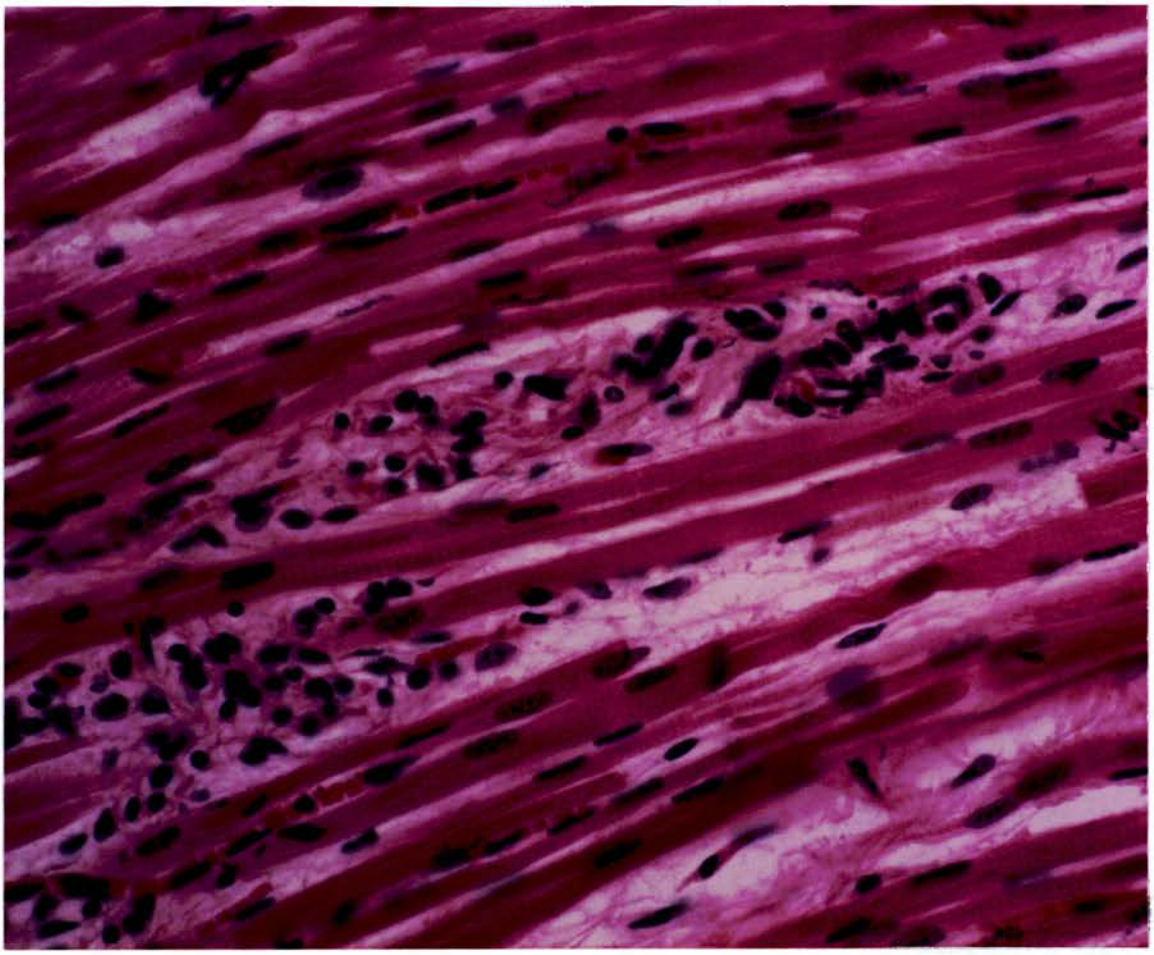

Fig 4-38 **Heart F889; multiple focal areas of myocardial necrosis with mixed inflammatory cell infiltration. x864.**

Where there was serological evidence of a concurrant B.V.D and I.B.R involvement in the abortion, there was an almost total absence of pathological changes in the tissues examined.

4.7. **Lesions recorded with no particular diagnostic associations.**

These may be listed:

i) erythroblastosis in fetal livers, which can be found in association with hepatomegally.

ii) conjunctival hyperplasia with goblet cell formation, together with an accompnying focal necrotic placentitis and amnionitis. Varible degrees of peri-bronchiolar cuffing can be present.

iii) oxalate crystals in a small number of kidney tubules located at the boundary between cortex and medulla.

iv) oedema of the chorio-allantoic membrane, accompanied by a narrow band of fibrocytes and mixed macrophages which infiltrate the stroma.

v) trophoblast hyperplasia.

vi) presence of mucous cells in epithelium covering some tips of the caruncular septae.

DISCUSSION.

Clinical spontaneous abortions are considered to be the "tip of the iceberg" of pregnancy wastage. Data from many animal species demonstrated that early fetal losses may be as high as 50% (Hendrickx and Binkherd 1980). Hawk and others (1955) have estimated that the embryonic mortality within herds which have had a previous history of infertility, could be as high as 52% in cattle fetuses up to 34 days gestation. Fosgate and Smith (1954) found that of 690 pregnancies between 34 days and full-term there was a fetal loss of 6.4%, a figure which is consistent with the findings in this study where 149 abortions occurred over a two-year period in an estimated cattle breeding population of 3660 animals, representing an annual abortion rate of 2% of fetuses with a crown-rump length greater than 21cm, ie. gestational age of approximately 100 days. The abortion rate recorded here is substantially higher than that published in M.A.F.F Veterinary Investigation Diagnosis Analysis (1986) for the United Kingdom; 32,496 abortions were reported out of a total breeding cattle population of 5.33 million (Meat and Live-stock Commission 1986), representing a figure of 0.7%.

Under-reporting of cattle abortions by farmers has long been suspected by veterinary authorities, and Murray (1984) has shown that for each abortion reported to the veterinary investigation service in Guelph, Ontario, Canada

3.4 fetuses were actually aborted on each farm. If the United Kingdom abortion rate was adjusted by this figure, it would be similar to the rate described in this study.

The proportion of abortions known to be associated with an infectious aetiology is unknown. In one study of those abortions where a diagnosis was determined, 90% were related to an infectious agent (Hubbert and others 1973). Any list of possible infectious causes of cattle abortion is only as accurate as the standards of the diagnostic organisation will allow. Efficiency in diagnosis depends on:-

a) laboratory methods of diagnosis and their accuracy
b) budget requirements to support diagnostic facilities, and availability
c) professional attitude of clinical and laboratory staff to problems
d) pressures applied to achieve a diagnosis (Nusbaum and others 1978).

All too commonly, serological findings that an aborting animal has been in contact with an organism is the only evidence that the organism caused the abortion. Similarly, the isolation of a microbial agent from an aborted fetus is accepted as evidence of a bacterial or viral cause. In this present study, a diagnosis of the cause of an abortion episode was based on pathological changes observed in either fetal or placental tissues, supported by a multi-level diagnostic approach, described by Kradel (1978).

There is believed to be no transplacental transfer of immunoglobulins from dam to fetus in cattle (Brambell 1970). Because the fetus is capable of making an immune response to several antigens, the detection of fetal antibodies in conjunction with other pathological findings has been found useful in attempting to determine the aetiology of bovine abortions (Miller and Wilkie 1979). However, Miller (1977) has pointed out that there are two possible courses by which foreign antigens can stimulate the fetal immune system; the haematogenous route from the placenta via the umbilical vein, and local invasion of the fetus through skin and mucous membranes in contact with contaminated amniotic fluid. Ellis and others (1978) found that there was a gradual rise in the proportion of normal fetuses containing immunoglobulins in their blood, from the fifth month of gestation (10% of samples) to term (50% of samples). The older the fetus, therefore, the more likely it was to have come into contact with antigen. Their additional finding that 70-80% of abortuses had immunoglobulin present in fetal fluid samples could suggest a similar conclusion, but the higher percentage of sero-positive fetuses in this group indicates that active infection could also be associated with this observation.

There is disagreement as to whether the immunoglobulin class most frequently detected in aborted fetuses is IgM (Miller and Quinn 1975; Ellis and others 1978), or IgG (Braun and others 1973; Sawyer and others 1973; Kirkbride

and others 1977). It has been suggested that immunoglobulins which are present in aborted fetuses in high concentration can inhibit the growth of bacteria in vitro, and be responsible for the relatively low recovery rates of microbial pathogens found by laboratories in aborted material (Logan and others 1981).

Of 124 fetuses examined in this study, immunoglobulins IgM and IgG were found in 23; IgG alone was found in 9 fetuses, and IgM in 3, a total of 35. Specific antibodies to either B.V.D. virus, I.B.R. virus, or L.hardjo were identified in 20 fetuses. Of these 35 cases, 28 (80%) presented histological lesions either in the fetus or the placenta. This finding is rather higher than the 61% recorded by Miller and Quinn (1975), but similar to the findings of Kirkbride and others (1977), and Ohmann (1981). In this study, accompanying fetal and placental lesions suggested a diagnosis in 19/35 cases.

Perhaps the most interesting findings here were in five fetuses where specific titres to B.V.D. virus were found but no IgM/IgG was present, and in two which had L.hardjo antibody but were IgM/IgG negative. Lesions were identified in placentomes from six cases (F 017;F 712;F 785;F 886;F 1224;F 1410), all of which had crown-rump length greater than 80cm, ie., gestational age over 230 days, characterised by villous necrosis and a predominantly mononuclear cell infiltration in septal and crypt tissue. Oedema, haemorrhage, fibrosis, and occasional mineralisation were additional variable

findings. Conjunctival hyperplasia was found in F 1224, and non-specific lesions were found concurrently in 4/6 lungs examined.

The diagnostic significance of immunoglobulins in fetal fluids should be assessed with caution. Low concentrations of IgM have been demonstrated in the serum of normal fetal lambs and calves after mid-gestation, as well as small quantities of IgG in serum from unsuckled newborn calves (Silverstein and Pendergast 1970). In the absence of demonstrable specific antibody, some of the IgG or IgM present may be either non-specific, the synthesis of incomplete immunoglobulin, or possible synthesis of natural antibody. In an abortion where maternal serology may suggest a likely causal agent but fetal immunoglobulins are absent, other influencing factors may need to be considered:-

a) the possible immuno-suppressive effect of natural corticosteroids present in fetuses and associated with parturition (Osburn 1973).

b) the immuno-suppressive effect of non-cytopathic strains of B.V.D virus upon the fetus (Scott and others 1973).

It can no longer be assumed that non-specific immunoglobulins found in aborted bovine fetuses are indicative of an infectious aetiology. It had been assumed that specific immunoglobulins present in aborted fetal fluids were of fetal origin and, therefore, significant. Poitras and others (1986) inflicted placental injuries upon pregnant ewes,and

and assessed the amount of leakage of an antibody against human red blood cells into the fetal circulation. They found that the severity of placental lesions did not predispose to leakage of maternal antibody into the fetus, even with the extensive caruncular lesions associated with A.fumigatus infection. Pathological changes such as necrosis of trophoblast and endometrial epithelium, a necrotising vasculitis of fetal villi and chorio-allantois, which destroy the normal close affiliation of maternal and fetal circulations at the feto-maternal interface, could allow transfer of maternal immunoglobulins to the fetus. For antibodies found in the fetus to be of value in reaching a diagnosis of abortion, it is essential to know their origin.

In this study, the pathological changes in fetus or placenta which were associated with either specific or combined infections diagnosed from fetal serology were relatively uniform. It was not possible to make a diagnosis solely on histological findings. In nine fetuses which were IgG/IgM positive with specific antibodies to B.V.D.in thoracic fluid (three of which presented serological evidence of concurrent L.hardjo infection), the pathological observations did not assist the diagnosis. There was a uniform mononuclear cell infiltration in fetal soft tissue organs examined, and evidence of chronic placentitis in those cases where this material was submitted. Of six cases where L.hardjo was diagnosed from positive maternal serological findings, four cases presented evidence of an

acute haemorrhagic placentitis. A number of the lesions could have resulted from non-fatal disease which affected the fetus or they could have been a response, independent of the precise causal agent of the abortion.

In future, it seems desirable that detection of specific fetal antibody to known infections should be supported by relevant lesions in fetus or placenta, and less reliance placed on finding non-specific fetal globulin.

The use of paired maternal serum samples taken from an aborting cow at the time of the initial visit, followed by a second sample at least four weeks later, is a common method of diagnosing abortion. The method is based on the detection of circulating antibodies against likely infectious agents. In general, a four-fold change in titre is assumed to be an accurate reflection of true antibody variation (Thrusfield 1986). Where Enzyme-linked Assay (E.I.A) methods are employed, a change of greater than 0.2 Optical Density units is taken as significant (Holliman 1987). Serum neutralisation (S.N.) is the most sensitive and commonly used serological examination for B.V.D.virus. Whilst it should be expected that a four-fold rise in maternal antibody titres is a necessary criterion to make a diagnosis, it is difficult to demonstrate in practice. This may come about because abortions associated with B.V.D.virus infection take place some time after the initial viral challenge, and paired sera usually reveal little since the antibody response is already complete (Kahrs 1968; Duffell and

Harkness 1985). S.N.titres of 1/10 and over can be regarded as significant.

Of the 39 abortions associated with B.V.D virus in this study, 17 showed a constant or rising maternal titre of 1/128 or above and 12 a four-fold change in the initial titre which ranged between 1/32 to 1/64. In 21 other cases the initial maternal titre was 1/32 or greater but no four-fold change in convalescent titres was present, and therefore an association with B.V.D infection could not be confirmed. In 14 of these 21 cases, routine fetal and/or placental sections were examined histologically; placental lesions similar to those in cases associated with B.V.D. infection were found in seven of these cases, and 6 cases gave comparable diagnostic lesions in either lung, liver, or eyelid. It would appear that animals with initial titres of 1/32 or 1/64, but with lesser changes in titres between the times of sampling, could possibly have aborted as a result of B.V.D. infection.

The general principles for the serological diagnosis of B.V.D virus infection also apply to I.B.R virus infection but epidemiological differences do exist between the two conditions which may influence the interpretation of laboratory findings. I.B.R virus has been described as causing an acute pyrexia in dairy cattle, with accompanying loss of appetite and milk yield. Morbidity rates of 20-75% have been experienced in affected herds (Anon. 1979). Unlike B.V.D infection which is often not fatal to the fetus other

than in early pregnancy, I.B.R infection is fatal; an acute septicaemia is characterised by focal necrosis of soft tissues (Kendrick and others 1971). After the pregnant cow and fetus have been exposed to the virus, abortion follows within 18-90 days (Kendrick 1973). The immune response to I.B.R infection can be gauged by the appearance of S.N. antibodies from the 14th day after infection (Smith and others 1978). Cows with S.N. titres as low as 1/3 may be protected against infection after exposure to the virus (Kendrick and others 1971). The virus does not survive in the plasma of cattle which contains S.N. antibodies to I.B.R.

Smith and others (1978) showed that by day 14 after I.B.R infection, 33% of animals had responded with titres of more than 1/16, and the remainder gave titres of less than 1/8. Flammini and Allegri (1972) described a field outbreak of stillbirths and neonatal death in cattle associated with I.B.R infection; out of 18 adult cattle that were blood sampled, only two animals produced S.N. titres of more than 1/16 to I.B.R. The majority (16/18) of titres were less than 1/8. It is apparent that S.N. antibody titres of more than 1/4, or an O.D. of more than 0.10 in E.I.A methods, may be significant (Holliman 1987).

Two serological surveys have been carried out in cattle populations, to determine the random percentages of I.B.R sero-positive samples. The first screening showed that 3.7% of serum samples were positive (Dawson and Darbyshire 1964)

and Kirby and others (1978) later gave a figure of 6.8%. Therefore, it is to be expected that the majority of cattle bled at random will provide negative titres to I.B.R virus.

In the present study, the serological criteria used as a basis for associating I.B.R infection with abortions were similar to those used for B.V.D infection. 21 abortions were associated with I.B.R virus infection, based upon both histological and serological methods. In three cases lesions were found which were suggestive of I.B.R infection but supportive serological evidence was not forthcoming; the initial titres were more than 0.1 O.D.units but a rising titre of more than 0.2 O.D units was not demonstrated. In 17 cases, aborted cows produced titres to I.B.R virus but they did not present a significant change in titre for the convalscent sample and, were classed as negative in the absence of fetal material suitable for pathological examination. Three cows had initial I.B.R titres of more than 1.0 O.D.units, and five cows had more than 0.1 O.D.units. If serological evidence is taken as the sole criterion for associating I.B.R. with abortion, the total number of such cases would be as high as 30, an incidence rate of 24%.

Extensive studies in the United Kingdom over the past decade into infection of cattle with Leptospires of the Hebdomadis group have clearly associated it with abortions (Little and others 1980; Hathaway and Little 1983b), with mastitis, and sudden drop in milk yield (Higgins and others

1980). It has been widely recognised that serological methods alone are inadequate for diagnosing this condition, but even greater difficulties exist in trying to isolate L.hardjo from field clinical material (Ellis and others 1976).

For a long time, veterinary practitioners have submitted blood samples from aborted cattle for routine diagnosis. Much work has been carried out to determine the relevance of serological testing for diagnosing leptospiral abortions, employing both complement fixation (C.F.T) and microscopic agglutination (M.A.T) tests to identify leptospiral antibodies.

Interpretation of these serological tests has proved unsatisfactory because:

i) although titres rise rapidly after initial bacterial challenge, when abortion occurs several weeks later there is then no a rise in titres (Hodges and Ris 1974).

ii) aborting cattle which have initial blood titres of more than 1/100 to L.hardjo are conventionally regarded as positive, but up to 22% of cattle aborting from this infection can show M.A.T titres of 1/10 or less (Ellis and others 1981).

iii) high titres associated with L.hardjo infection are not common place and M.A.T titres of 1/40-1/50 should be read as positive, rather than the arbitrary selection of 1/100 which is the current standard

(Little and others 1980).

iv) renal carriers of the disease may give titres as low as 1/10, but be still excreting the organism (Ellis and others 1981).

v) there is no titre for L.hardjo antibodies which positively indicates that the organism is the cause of abortion in dairy cattle (Elder and others 1985).

Titres of 1/400 and over, which were suggestive of recent infection in cattle which had aborted (Higgins and others 1980; Hathaway and Little 1983b), were taken as positive in this study. Titres of 1/100 were interpreted as being associated with herd background infection. 16 cases produced M.A.T titres of more than 1/100; a further two cases that originated from a farm with known L.hardjo infection and were included in an abortion storm involving nine pregnant cattle, gave completely negative titres but fetal and placental lesions were consistent with those in other L.hardjo positive cases. In 16 cases, abortion could be ascribed to recent infection with L.hardjo , and in a further 17 cases a back-ground infection was present in the herds involved.

Lesions in skin and conjunctiva.

Under certain circumstances, amniotic fluid contains micro-organisms for a sufficient length of time to allow a reaction to take place between them and the fetus before death. Fetal cutaneous, gastrointestinal, and pulmonary

lesions can be expected as a direct result of contamination with infected amniotic fluid (Wigglesworth 1984a). Gross cutaneous lesions were not commonly found in this study, but when present they were associated with a concurrent placentitis and with fungal infections. These skin lesions did not fit the description given by Canant (1985), although their distribution was similar. Histological examination of 118 sections of eyelid confirmed the diagnosis of cutaneous fungal infection in only three cases, from the mixed inflammatory cells, exudate, and fungal elements found on the skin surface.

Lind and Hytten (1972) showed that there is considerable fluid exchange through the unkeratinised skin of the developing human fetus before mid-gestation. It has been suggested by Hubbert (1974) that the presence of keratinised skin could reduce the movement of fluid between the amniotic and fetal compartments in normal bovine pregnancies, the direction of flow depending on the osmotic pressure of fluid in either compartment. As keratinisation of skin increases with fetal age the permiability of skin should decrease. It was concluded that if fetal death occurred after 200 days gestation, subcutaneous oedema should be a common finding; fetal mummification accompanied by fluid resorption should be more common with deaths during early pregnancy, when fewer layers of keratinised skin cover the calf.

Because fetal death often occurs several days prior to abortion, generalised autolysis is common. When this occurs,

the skin undergoes changes such as seperation and displacement of epidermis from corium or "slipping", sloughing, and dehydration. Subcutaneous changes may include sero-sanguinous fluid accumulation and tissue discolouration, which may mask oedema, making it difficult to interpret gross findings. In 24 fetuses showing evidence of early autolysis there was gross and histopathological pulmonary inter-lobular oedema; this finding may indicate generalised oedema in the fetus. Histological sections of eyelid, which were routinely taken through the muco-cutaneous junction, did not present any evidence of oedema in fetuses over 200 days gestation; sections obtained from mummified fetuses did show the early developmental stages of fetal skin by the presence of hair follicles, reduction in numbers of epidermal cell layers, and the absence of keratin, even though there was a loss of cellular detail.

The crown/rump length of the seven mummified fetuses found in this study ranged from 19 to 33 cm, representing gestational ages from 100 to 135 days. Of 118 aborted fetuses which were not mummified, only six had C/R lengths within a similar range. It would appear unlikely that the absence of keratinised epidermal cells could determine whether dehydration and mummification should follow fetal death. Other relevant factors must include the degree of placental inflammation or endometrial damage experienced at the time of fetal death as this may adversely influence immunological and endocrine relationships between fetus and

dam. Should the corpus luteum of pregnancy persist in spite of fetal death, mummification could be the end result, brought about by loss of fetal fluids to the amniotic compartment through structurally immature skin and hence reabsorption through the fetal membranes and uterine wall into the maternal circulation. However, if fetal death is accompanied by caruncular and/or endometrial inflammation which provoke prostaglandin production and its release into the maternal blood stream, luteolysis of the corpus luteum will occur, followed by withdrawal of progesterone which is necessary to maintain pregnancy (Garfield and others 1982), resulting in the abortion of a relatively fresh fetus.

Histological lesions of the conjunctiva were more frequent, and may be grouped into two categories. The first and most common was non-specific inflammatory cell infiltration of the sub-epithelial connective tissue. Fetal bacterial infections, associated with an acute placentitis followed by a subsequent spread of infection through the chorion, mesenchyme, allantois and amnion, to the amniotic fluid were accompanied by a polymorphonuclear infiltration. Under these circumstances, the fetus is surrounded by an environment containing infective organisms; hence pure bacterial cultures of C.pyogenes were isolated from fetal lung and stomach contents in several cases. This is similar to the experiences of Miller and Quinn (1975).

Mononuclear cell infiltration appeared to follow haematogenous spread of viral and L.hardjo infections to the

fetus, varying in intensity depending on the duration of exposure to infection prior to fetal death. This conclusion is made from placental lesions, high initial maternal serum titres, and degrees of change in convalescent titres. Sub-conjunctival mononuclear inflammatory lesions have been observed in infections with I.B.R (Miller and others 1978), as well as with B.V.D (Ohmann 1982), and U.diversum (Miller 1984).

The second and less common lesion in the conjunctiva was epithelial hyperplasia and goblet cell proliferation, not associated with any specific infectious organism. These changes were observed in 9/118 fetuses, and usually were accompanied by sub-epithelial lymphocyte infiltrations. Goblet cell hyperplasia is a most uncommon lesion compared with squamous metaplasia (Wilcock 1985), whereas lymphocytic infiltration is characteristic of a non-specific response to chronic antigenic stimulation. This pathological picture might indicate that the amniotic fluid may contain a potential pathogen which is capable of provoking chronic conjunctival irritation, because it was always accompanied by a placentitis and/or amnionitis.

Infectious causes of conjunctivitis in calves or yearling cattle include B.V.D, Malignant Catarrhal Fever, and I.B.R. viruses (Wilcock 1985), as well as Listeria monocytogenes (Morgan 1977), Moraxella bovis (Wilcox 1970), and Mycoplasma spp (Langford and Dorward 1969). Vitamin A deficiency has been described as a predisposing factor by

El-Sanousi and others (1978). Whilst the pathology of these conditions has been well documented, the specific conjunctival lesions in nine fetuses described here have not been previously reported. Miller (1984) has recorded similar lesions in sections of fetal eyelid to those found and illustrated in this study which were associated with experimental U.diversum infection in pregnant cattle; reference slides are available to support his description (Slide 78. Pathology of Reproduction: University of Guelph. Ontario). This organism has been shown to be associated with abortion and the birth of weakly, full term calves, and is akin to infections in pregnant women with T-strain Mycoplasma, which also cause abortion (Miller and others 1983c). Such infections are frequently persistent.

Heart lesions.

Epicardial haemorrhage was the most common gross lesion, found in 18/118 fetuses examined. Ecchymoses or petechiae were located along the line of the coronary vessels or on the ventricles, frequently accompanied by congestion and haemorrhage in lung and liver. The complete pathological picture was characteristic of acute fetal asphyxia (Wigglesworth 1984b). Microscopic evidence of inhalation of meconium or squamous cells into the pulmonary air-ways in these cases appears to support this opinion, even though obvious meconium staining of fetal skin and hair was absent in some cases. This lesion appeared to be associated with bacterial infections in aborted fetuses, and

is in keeping with the opinion that sub-epicardial haemorrhagic lesions are common in many cases of acute infectious fevers (Anderson 1976).

In human medicine, the haemorrhagic features of acute asphyxia are exaggerated in infants who die following premature placental separation; lesions are extensive in distribution and large in size (Wigglesworth 1984b). This present study did not compare the size of haemorrhages. Whether these lesions are a pathological change arising from early placental separation, a placental response to infection, or a fetal reaction to bacterial or fungal infection, can only be resolved by balancing the pathological evidence from the abortus and its placenta.

Although heart pallor was a gross feature in eight cases, histological examination of tissues failed to relate this to any specific myocardial lesion. Wigglesworth (1984b) has commented on the difficulty of recognising early myocardial necrosis in fetal hearts, since myocardial fibres are small and normal nuclei may appear pyknotic. It appears reasonable to suppose that ischaemic myocardial necrosis is common, but evidence for this ought to be supported by lesions suggestive of inadequate blood perfusion throughout other visceral organs. However, autolysis is such a general feature in bovine abortion that the gross observations of heart pallor in this study could arise from this process alone.

Microscopic diffuse non-specific myocardial degener-

ation and non-specific myocarditis were encountered in 26 fetuses. The basic pattern was usually a mononuclear cell infiltration in interstitial and/or peri-vascular connective tissue, with occasional oedema. This low incidence is surprising when compared with that in young or adult cattle, where pathological changes of the heart are common in viral, bacterial, or fungal septicaemias (Robinson and Maxie 1985).

Focal myofibril necrosis surrounded by inflammatory cells has been recorded after experimental infection of bovine fetuses with Chlamydia (Storz and Whitman 1981) and following infection with I.B.R (Miller and Quinn 1975). This lesion was associated with antibodies in maternal serum to I.B.R in five cases, and to B.V.D in three cases. The histological changes were the same: a myocytolysis or patchy necrotic foci was accompanied by a predominantly mononuclear cell infiltration. Variable interstitial oedema sometimes surrounded individual myofibrils.

The lesions described here could cause the fetus only mild and transient illness. The absence of multi-nucleated giant cells or widespread fibrosis indicated that no progressive myocardial disease was present. Any inflammatory lesions which developed during the acute stages of disease could have resolved, leaving behind little residual evidence of cellular changes. Alternatively, since there is often a delay of a few days between onset of extra-cardiac viral infection and the appearance of myocarditis, the lesions observed would vary according to the length of time from

initial infection to fetal death, and in acute infections such as I.B.R. pathological changes might have had little time to develop.

It is uncertain how some viruses cause myocardial damage; there may be a direct viral cytotoxic effect, or a cell-mediated immune reaction which destroys those myofibrils containing virus or viral-dictated antigens. Accumulated evidence favours the second hypothesis, since myocardial inflammatory cell infiltrations are characteristic of most viral diseases (Woodruff 1980).

Whilst non-specific myocardial lesions were demonstrated in a further five fetuses, no supporting evidence was available to indicate the aetiology.

Lung lesions.

The high frequency with which the pre- and neo-natal respiratory system is affected by disease reflects the importance of fetal lung development and function in determining the eventual outcome of pregnancy. In one hospital survey, Helwig (1933) found that 41.5% of lungs from stillborn fetuses exhibited some degree of pneumonic change. The lung is a prime target for generalised disease processes because of its intimate relationship with the amniotic fluid which surrounds the fetus, its vulnerability to biochemical changes during the course of embryological development, its rich blood capillary network, and the influence of hormones on lung maturation.

Over half of the fetal lungs examined in this study

presented microscopic lesions; somewhat fewer showed any gross morphological lesions, usually inter-lobular oedema.

Microscopic pulmonary oedema or congestion with minimal inflammatory cell infiltration was seen in lungs of 6/65 fetuses. No association could be made with infectious causes of abortion but three cases (F 1347;F 1388; F 1492) had coincidental findings of focal necrotic placentitis and conjunctival hyperplasia. Liver congestion was a feature in a further two cases, but no cardiac abnormalities were found. Oedema and congestion can arise from increased hydrostatic pressure, similar to that encountered in endotoxic and surgical "shock", leading to congestive heart or renal failure. This would produce fetal hypoxia, carbon dioxide retention, with consequent acidosis, pulmonary vaso-constriction, and pulmonary hypoperfusion. The end result is capillary endothelial damage, which then causes leakage of plasma into interstitial and inter-lobular tissues (Boyle and Oh 1973). In premature infants and lambs, born alive, the sequelae to this process is the syndrome of Hyaline Membrane Disease (Liggins and Howie 1972), but the typical pathological lesions seen in this condition are never found either in still-born infants or in those which die within a few hours after birth (Robbins and others 1984). Reynolds (1970) was unable to produce the disease experimentally in mature lamb fetuses, except in one case of severe asphyxia which led to cardiac arrest before delivery.

In the absence of other lung lesions in these six

fetuses, minimal inflammatory cell infiltration, and the finding of skin cells or meconium within the airways, Fetal Distress Syndrome should be considered as a contributory factor in these fetal deaths, caused by factors as yet undetermined (Miller 1981). In coming to this conclusion, account must be taken of the problems that exist when interpreting such a histological picture. For instance, the diagnosis of fetal pneumonia is more difficult than in the adult animal. Reasons for confusion are:

i) unexpanded alveoli lined by cuboidal epithelium
ii) numerous immature cells in blood capillaries
iii) presence of squamous cells, amniotic fluid, and meconium in airways.

The mere presence of scattered intra-alveolar or inter-bronchial leucocytes and/or mononuclear cells is unlikely to constitute an early pneumonic lesion; it may simply represent a minimal response to amniotic debris in the lungs (Penner and McInnes 1955). The same authors are clear that aspiration of amniotic fluid into the airways is not related to the incidence of fetal or neo-natal pneumonia, even though it is a common histological feature at autopsy. In lungs from 65 human fetuses examined post mortem, they located meconium or cornified epithelial cells in the airways of 68% of lung sections.

In an experimental study of the effects of anoxia on bovine fetuses, Dufty and Sloss (1977) observed that the fetus commonly released meconium; the degree of skin

staining varied with the experimental procedures. They suggested that meconium release was an indication of fetal hypoxia or anoxia, and was likely to be the result of premature placental separation from the caruncle. A similar conclusion was made by Walker (1954) after finding meconium in human fetal airways; he dubbed the condition "fetal distress". In these cases, the oxygen saturation of blood in the umbilical vein was at or below 30%. Mandelbaum (1973) said that meconium release in human fetuses was the result of relaxation of the anal sphincter and an increase in intestinal peristalsis, both a response to anoxia; an increase in carbon dioxide tension and a lowering of the blood pH could exert a similar effect. Hams (1973) extended the argument by saying that normally weak fetal respiratory movements could be stimulated by anoxia into strong inhalation, and that meconium with other amniotic debris could thus be inhaled into airways, resulting in a fetal pneumonia.

Beside premature placental separation, Miller (1981) listed other causes of bovine fetal hypoxia which may produce a similar histological picture:

i) placentitis

ii) maternal circulatory hypoxia as a result of maternal disease, eg.pneumonia

iii) impaired delivery of oxygen from placenta to fetus eg.umbilical cord compression

iv) umbilical cord abnormalities

v) fetal cardio-vascular problems

vi) prolonged dystocia

Umbilical cord lesions have been described as causing fetal hypoxia in the horse (Whitwell 1980). Whitwell and Jeffcott (1975) noted an excessive twisting of either amniotic or allantoic portions of the umbilicus. Cords from these cases were found to be longer than the accepted normal length of 36-83cm. (In the horse, the umbilical cord is slightly over half the length of the mature fetus). Cord oedema and haemorrhage, fetal autolysis, subcutaneous oedema and excessive fluid within serous cavities were recorded. There were no similar gross cord lesions found in this study. There are three possible reasons for this:

i) spontaneous rupture of the cord normally occurs at, or within minutes of, birth

ii) there was a high incidence of retained fetal membranes after abortions

(Both these reasons prevented a complete examination of fetus, intact cord and placenta.)

iii) normal umbilical cords in cattle are 21-45cm long at term (Fleming 1930). The C/R length of a full-term fetus is around 90-100cm (Evans and Sack 1973), somewhat shorter than in the horse. The relatively short cord in cattle may be the reason why cord lesions are rarely observed.

The present study has indicated that the most consistent factor associated with inhalation of meconium and

amniotic debris by the bovine fetus, which occurred in 35/43 abortuses, was a concurrent placentitis. In 2/43 cases this, together with a non-specific interstitial inflammatory cell infiltration was the only fetal lesion present; in both these cases there was maternal serological evidence of B.V.D. infection.

Penner and McInnes (1955) have classified fetal pneumonia on the histological changes found in 71 pre- and full-term stillborn infants:

i) diffuse acute: minimal or no bronchial involvement. Intra-alveolar and interstitial infiltration of lung parenchyma by polymorphonuclear leucocytes. The infiltration is diffuse but may vary in intensity. Few mononuclear cells are present.

ii) diffuse pneumonia with mononuclear cell infiltration: the cellular infiltration is predominantly mononuclear within the lung parenchyma. Very little amniotic fluid is present in the airways, but a few inflammatory cells can be located within them.

iii) bronchial and peri-bronchial pneumonia: the lumina of large and small airways are stuffed with polymorphonuclear leucocytes, and alveoli are surrounded by patchy intra-alveolar polymorphonuclear infiltration. Interstitial tissue is infiltrated with varying numbers of acute and subacute inflammatory cells.

Miller (1981) has opined that if the correct description of specific lung lesions were given in all

abortion investigations, it would help in determining the likely method of spread of infections from dam to fetus. For instance, a suppurative broncho-pneumonia may develop as a consequence of bacteria entering the fetal lungs as contaminated amniotic fluid is inhaled. Again, if an infectious organism, eg.,PI3 virus, spreads via the fetal circulation an interstitial pneumonia may develop (Swift 1973). However, it is very difficult to associate a specific histological picture with any one agent causing abortion.

The relevance of bacteria grown from fetal lung cultures has been addressed by Penner and McInnes (1955). They found that most diseased and normal lungs produced common bacteria. In the normal lungs of 88% of fetuses which died at birth or were stillborn and made up the control cases in the study, bacteria were seldom seen in the prepared histological sections whereas they were present within inflammatory exudate in the airways of pneumonic fetal lungs. It was concluded that bacteriological findings without histopathological support must be treated with caution. The presence of bacterial clumps in airways, accompanied by inflammatory cell debris, may be a much more significant pathological finding.

Broncho-pneumonia was found in 24/65 lungs from aborted fetuses in this study. 16 of these cases were related to a concurrent bacterial placentitis, C.pyogenes, E.coli, and fungi being the predominant organisms. The remainder were associated with mixed bacterial cultures from lung and

placenta, together with various non-specific inflammatory changes in lung parenchyma. These findings indicate a much greater incidence of bacterial broncho-pneumonia in cattle abortions than in infant abortion and stillbirths. Penner and McInnes (1955) found a 5% incidence in human abortions compared to an overall rate of 16% in this study. This difference may be explained by the fact that only a very small proportion of spontaneous human abortions are caused by infection (Rushton, in press).

Diffuse interstitial pneumonia occurred in 29/65 lungs, with a predominantly mononuclear inflammatory cell infiltration in the lung parenchyma. Amniotic debris was frequently present but was considered to be insignificant. In addition, there were related serological or pathological findings in a number of cases:

i) antibody to B.V.D in fetal thoracic fluid or maternal serum (12/29 cases).

ii) antibody to L.hardjo in fetal thoracic fluid or maternal serum (7/29 cases).

iii) antibody to I.B.R in fetal thoracic fluid or maternal serum (2/29 cases).

iv) fetal IgG/IgM in thoracic fluid with no other significant findings (3/29 cases).

iv) epithelial hyperplasia in fetal conjunctiva (2/29 cases).

Interstitial pneumonia was reported from 5/50 aborted fetuses examined by Miller and Quinn (1975), that were

characterised by extensive peri-bronchial lymphoid accumulations; they thought that this lesion could be associated with PI3 virus infection as described by Swift and Kennedy (1972), but serological examination of aborted fetuses was necessary to resolve the precise aetiology.

From the present study, it appears that there is a positive relationship between diffuse interstitial pneumonia in aborted fetuses and serological evidence of a concurrent infection with either B.V.D and/or I.B.R virus in the dam, or in the fetus. There is a similar association with L.hardjo infection. All three organisms spread through the fetus by the blood and the lung seems to be particularly sensitive to systematic disturbances that involve it.

Liver lesions.

The structure and function of the liver account for the frequency of lesions which are not directly of hepatic origin. Pathological changes in the liver are much more common than is hepatic failure, these lesions being important because of the insight they give as to the nature of many systemic diseases (Kelly 1985).

The fetal liver is structurally and functionally different from that found in the adult or neo-nate. It is larger relative to the size of the body, and is physiologically immature. The relatively greater size is due partly to persistence of extra-medullary haematopoiesis in that organ. Many of the functions of the fetal liver are only marginally adequate to carry out the demands placed

upon them, deficiencies of bilirubin glucuronyl transferase, hydroxylating enzymes and protein synthetic capacity being characteristic examples (Robbins and others 1984).

It is against this background that the fetal liver's response to insult and injury has to be viewed. For instance, in the face of bacterial or viral infection the mid-term fetus will respond immunologically with an appropriate polymorphonuclear or lymphocytic leucocytosis. If the infection is haematogenous, the involvement of the liver may be restricted to pathological changes around the portal triads. However, infiltrating lymphocytes with their dense, round nucleus and relatively sparce cytoplasm may easily be mistaken for normal clusters of nucleated pro-erythroblasts found in sinusoids of normal fetal liver. These haemopoietic cells are numerous in the second trimester but the numbers fall as full-term is approached (Wigglesworth 1984c).

Erythroblastosis occurs in infants. It is characterised by an enlarged liver with massive extra-medullary erythro-poiesis. The parenchymal cell plates may appear disrupted by the masses of haemopoietic cells. The causes of this condition have been listed as:

i) severe rhesus iso-immunisation syndrome.

ii) fetal haemolytic anaemia.

iii) infections, including cytomegalovirus.

iv) fetal hydrops.

(Langley 1973).

Histologically, the diagnosis of this condition depends on the identification of abnormally increased erythropoietic activity in the infant, with increased numbers of reticulocytes, normoblasts, and erythroblasts in the circulating blood. The largest of these immature cells tend to become trapped in the pulmonary capillaries. Evidence of subcutaneous and visceral oedema may be found along with the hydrops syndrome, but also with generalised fluid accumulations in the body cavities. Additional changes may often exist together in the placenta, the stroma of chorionic villi being spread apart by oedema and the presence of large, round-to-polygonal, epithelium-like Hofbauer cells (Robbins and others 1984).

Whilst eight cases of erythroblastosis were recorded in this series, there are very few readily available reports in the veterinary literature which can confirm these observations or be used to make a critical appraisal of them. The clinical features in case F309 were fetal ascites and subcutaneous oedema around the head, neck, chest and fore-limbs. Meconium stains were found on the skin of the hind limbs and upper thighs. The gross weight of the liver was 2kg. Interlobular oedema was a gross and microscopic feature of the fetal lungs. Laboratory examination of fetal and maternal samples proved of no assistance in establishing a possible diagnosis.

In human medicine, erythroblastosis fetalis may be defined as a haemolytic disease in the newborn caused by

blood-group incompatability between mother and child (Robbins and others 1984). This clinical entity has been recognised in equines (Rossdale and Ricketts 1982), but not in cattle because of differences in the structure of the feto-maternal junction. In normal bovine pregnancy, there is no transplacental passage of fetal erythrocytes into the maternal circulation and no movement of maternal antibody into the fetus. As argued earlier in the discussion, in pregnancies put at risk by placental damage, transplacental leakage of maternal immunoglobulins into the fetus does occur. An opposing concept, that bovine fetal red cells could enter maternal capillaries through uterine epithelium and connective tissue stroma is difficult to accept, even if the normal integrity at the feto-maternal interface was interrupted by disease; endometrial stroma and epithelium do not appear to be important in the development of ruminant placental infection particularly by B.abortus (Anderson and others 1986a), and remain intact.

The present study suggests an alternative hypothesis for the development of this lesion, involving fetal intravascular haemolysis followed by sequential hypoxia, resultant injury to the heart and liver, circulatory failure and oedema which may be of sufficient duration and severity to cause fetal ascites. Those fetuses which did not develop oedema may have experienced a more acute syndrome.

Support for this is based on two pieces of pathological evidence. The first is histological, and comes from

the presence of widespread congestion in both caruncle and fetal lung with extra-vascular leakage of haemolysed blood in chorionic villi, present in the five cases reviewed. Fetal deaths all appeared to be caused by anoxia. Recognised aetiologies associated with this picture include L.pomona infection with a resultant fetal haemolytic anaemia (Murphy and Jenson 1969), nitrate/nitrite poisoning leading to the formation of methaemoglobinaemia in the dam (Winter and Hokanson 1964). In this latter condition, no significant pathologic changes were found in fetal or placental tissues except for signs of erythropoietic stimulation in calves born to affected dams. The second is serological, and supports the idea of leptospiral involvement. Maternal titres to L.hardjo were as high as 1/1600, with fetal titres to B.V.D at 1/64, and this suggests that these abortions were associated with acute leptospiral infection of the pregnant cattle within the previous 4-6 weeks. Thus, fetal anoxia could have ensued from anaemia, brought about by leptospiral haemolysins, which produced erythroblastosis.

The most common lesions in livers of aborted fetuses were non-specific inflammatory cell infiltrations around the portal tracts, and generalised congestion. Perinatal infections often provoke non-specific changes in the liver that suggest an infectious aetiology, but not its precise nature (Wigglesworth 1984c). When an infectious organism spreads to the fetus via the circulation, the first organ encountered will be the liver; hence, periportal inflamm-

atory lesions may indicate a haematogenous infection.

Autolysis was a frequent post mortem finding which most probably masked specific lesions, such as coagulation necrosis associated with I.B.R infection (Kennedy and Richards 1964), or suppurative hepatitis as seen in some cases of bacterial placentitis (Miller 1981) which were rarely encountered. This was disappointing, given the numerous accounts of hepatopathies experimentally induced by infectious causes of abortion, such as I.B.R (Kennedy and Richards 1964), L.monocytogenes (Njoku and others 1972; 1973a), Chlamydia spp (Storz and Whitman 1981), B.abortus (Anderson and others 1986a), and B.licheniformis (Mitchell and Barton 1986).

Placental lesions.

Fox (1978) has stated that the pathological examination of the placenta should be an integral part of the investigation on a stillborn infant. It is over-optimistic to expect such an examination to yield a cause, except in a minority of cases. What the pathologist has to do is elucidate the cause of death from the changes observed, which may frequently be misleading except in material obtained from recently-dead fetuses. This comes about because the placenta remains fully viable after fetal death, and so it may undergo morphological changes which are sufficiently marked as to invalidate histological findings; for instance, villous stroma becomes increasingly dense and fibrotic, and fetal villous blood vessels undergo

progressive sclerosis. Oedema is not uncommon after fetal death. There is little evidence to suggest that calcification occurs as post mortem change, although scattered calcification of terminal villi are commonly seen with macerated fetuses. The pathogenesis of these changes is reasonably clear, since the death of the fetus is followed by collapse of the fetal circulation through the placenta, which leads to fibro-muscular sclerosis of fetal stem arteries and stromal fibrosis. Villous oedema is probably due to accumulation of fluid transferred across the trophoblast from a compensating maternal circulation, and villous calcification may reflect a concentration of transferred calcium.

These changes may allow false conclusions to be drawn: first that the changes observed were present at the time of fetal death and are therefore partially responsible for the lost pregnancy; secondly that histological changes recorded in natural and experimental infections are authoritatively described and little account is given of normal post mortem changes.

Despite extensive arguments in the past, many medical pathologists who are interested in placental disease now feel that most inflammatory changes in the placenta are not due to hypoxia or idiopathic causes, but are brought about by infectious organisms which reach the placenta by transplacental or the haematogenous route (Perrin and Sander 1984).

Serur and Bayoumi (1979) carried out a histopathological study of retained (R.F.M) and spontaneously delivered placenta in cattle, unrelated to abortion. The microscopic lesions in cases of R.F.M and abortion should be similar; if any bacteria isolated had originated from an ascending infection via an open cervix, the resulting inflammatory changes should be the same. In fact, necrosis and desquamation of chorionic villi were predominant. Villous arterioles were dilated, hyperaemic, and showed early hyaline degeneration in their walls. 64/149 cases produced a mixed inflammatory cell exudate. Connective tissue oedema was also common. It was difficult to be dogmatic as to whether the cellular infiltration of inflammatory cells represented a response to infection entering the uterus before and during parturition as suggested by Rosenberger and Tilimann (1978), or was part of the aetiology of R.F.M, with an open cervix which allows ascending bacteria to spread into the uterus and its contents, associated with abortion (Paisley and others 1986).

Significant histological findings are the presence of bacteria within trophoblast cells and within inflammatory cell infiltrates (Anderson and others 1986a), or the formation of chorionic microabscesses (Altshuler 1984). Furthermore, the more severe the placental inflammation, the more likely is the fetus to succumb with a pneumonia or generalised sepsis.

There is yet another morphological problem to consider,

which is the presence of mononuclear inflammatory cells in the villous core of normal and diseased placentae. Enriquez-Yap (1974) described in detail the histological structure of normal placentomes in Bubalus bubalis (Philippine carabao). She noted the connective tissue elements of the villous core, which included dense, small, round-nucleated cells, similar to lymphocytes. Similarly, she gave details of the structure of the maternal septum; there was a connective tissue core, composed of varying amounts of collagenous fibres, together with connective tissue cells such as fibroblasts, undifferentiated mesenchymal cells, and numerous lymphocytes. Similar observations have been made concerning the structure of the normal placental bed in early pregnancy in humans, where the spiral arteries are surrounded by fibrinoid material and the adjacent decidua contains patchy but diffuse infiltrates of small, round cells (Rushton 1984). This "syncytial endometritis" is considered to be part of the normal adaptive process taking place in the placenta during pregnancy.

Generally, haematogenous infections involve the placental parenchyma itself rather than the membranes, and the inflammatory response to this is known as villitis. The inflammation is purely of fetal origin and maternal cells are not involved. Altshuler and Russell (1975) have segregated the villitides of human pregnancy into various histological groups:

i) proliferative villitis: inflammatory cells present

but no tissue necrosis.

ii) necrotising villitis: inflammatory cells present with intra-villous necrosis.

iii) reparative villitis: inflammatory process within the villi is undergoing organisation and repair.

In the bovine caruncle, maternal septal parenchyma also responds to infection with a marked inflammatory reaction. Both neutrophils and lymphocytes can infiltrate into the tissue which may also become oedematous. A septal peri-vascular leucocytosis, fibrin deposits, and presence of blood platelets may be indicative of severe vasculitis. These early changes may then progress to a suppurative endo-metritis, ulceration of the endometrium, and necrosis with lymphoid cell aggregations in the endometrial lamina propria (Anderson and others 1986 a:b).

In this work, the prevalence of septal lesions could be assessed only in sections of caruncle, whereas lesions of villitis were better appreciated in sections of cotyledonary chorio-allantois.

The results obtained in this study should be put into perspective. The mere recording of histological changes should not be presumed indicative of any absolute diagnostic significance. Account must also be taken of the clinical nature of the investigative procedures used, since it limited the amount of material that could be examined, such as taking caruncles from a number of different sites in the post-partum uterus. Financial considerations also prevented

the preparation of several blocks from distinct parts of the single placentome; such extensive investigations would have produced a more accurate account of the pathological changes. Finally, the cervix did not remain open and dilated sufficiently long enough after abortion to allow the removal of placentomes in the majority of cases, which further diminished the value of the placentome in elucidating the aetiology of abortions. Histological sections were made from 39 placentomes collected, all of which showed necrotic changes in trophoblast cells. Gross necrotic changes were found in 26 cases, and in 9 of these 26, there was evidence of haemorrhage and/or oedema.

Despite these shortcomings, histological examination of the placentome is a useful tool in abortion diagnosis. The pathologist can study the relationship between adjacent fetal and maternal tissues, in the context of the clinical background and history of the abortion episode. Examples can be made by examining cases F712 and F1796. The fetus of F712 died during premature delivery. Fetal lesions were aortic and epicardial haemorrhage with congestion of the liver. In the placentome there was villous necrosis and mineralisation, without any significant bacteriological findings. The placental lesion may be interpreted as post-mortem change, but there was in addition an infiltration of mononuclear cells within the caruncular septa, possibly indicating a non-specific endometritis in response to fetal trophoblast disease. If this had been due solely to post

mortem change, it would have been unusual to find a significant IgG concentration of 7mg/ml in fetal thoracic fluid together with a B.V.D titre of 1/8. This serological evidence is suggestive of a possible B.V.D challenge to the fetus some weeks prior to death, because specific sero-conversion had taken place. It is likely that the villous necrosis and calcification was a result of progressive fetal hypoxia, the gross fetal lesions lending weight to the hypothesis. The lymphocyte infiltration in the caruncular septa could have arisen because of chronic inflammatory changes at the feto-maternal interface which initiated fetal hypoxic changes. The fact that fetal death occurred during delivery makes the pathological findings a significant record of the events which surrounded that abortion.

For comparison, the fetus of F1796 was grossly auto-lysed, and placental changes were widespread necrosis and a coexisting polymorph inflammatory cell infiltration. A mixed bacterial culture was isolated from fetal stomach, lungs, and placenta. Neither fetal nor maternal samples produced any significant serological results. Histologically, massive numbers of bacteria were surrounded by inflammatory cell debris and necrotic tissue in the cotyledon but in the placentome, maternal tissue reaction appeared to be minimal with a mild leucocyte infiltration of the caruncular septa. The uterine epithelium remained intact. The interpretation of the histological findings in this case was of secondary bacterial invasion of the fetus after death, followed by

post mortem changes in fetus and placenta. It was not possible to associate a cause with this abortion because of extensive post mortem changes which masked any lesions that may have been present. It was estimated that the interval between fetal death and expulsion of the fetus was in the region of 4-10 days.

A false impression could have been given of the significance of the bacteria isolated from aborted material in this case, if the pathological findings in the cotyledon alone was used to make a judgement. It was with a similar subjective approach that the lesions found in other cases, both in the placentome and cotyledon, have been discussed.

The basic lesions in the placentome that were associated with bacterial infection were a diffuse necrotic villitis and polymorphonuclear cell infiltration in the caruncular septa. Necrosis of endometrial epithelium was a particular feature of C.pyogenes involvement, but otherwise the lesions were essentially similar to those found with experimental C.pyogenes abortions in sheep (Smith and others 1971). In case F886, micro-abscesses were located within connective tissue stroma at the base of the caruncle; bacterial foci wewre surrounded by large numbers of polymorph cells and fibrocytes; E.coli was isolated in pure culture from placentome, fetal stomach, and lung.

The pathology became harder to interpret in sections of placentome when necrosis and leucocyte infiltrations were accompanied by severe congestion in the septal stroma, and

when frank haemorrhage was found at the epithelial interface between trophoblast and endometrium at the base of the cotyledonary villi. In two cases from which fungi were cultured, extensive areas of haemorrhage and necrosis were well demarcated. In five other cases where abortion was associated with a positive maternal or fetal titre to I.B.R virus, a concurrent vasculitis was present in cotyledonary villi and caruncular connective tissue stroma. In both examples, irrespective of aetiology, the lesions indicated that severe damage had been inflicted on fetal and maternal blood vessels. This may have come about by direct invasion and destruction of capillary endothelium and thrombus formation in fungal infections (Hill and others 1971), or by vascular endothelial necrosis with inflammatory cell infiltration at the site of damage in I.B.R infections (Owen and others 1968).

The subtle placental lesions associated with I.B.R infection are likely to occur as a result of hypoxia, since oedema and some mineralisation become more obvious as the period lengthens between fetal infection, death, and abortion; this time period may be gauged from serological findings. It was not possible from this study to determine whether these lesions were the direct result of viral invasion of the placenta, or the result of post mortem change. According to Kendrick and others (1971), the placental changes in cattle following experimental infection with I.B.R virus and subsequent abortion were no different

from those found in cattle which had calved normally and were not infected. However, virus was present in placental tissues and it was concluded that inflammatory lesions could be absent in acute infections; insufficient evidence was available to show whether I.B.R virus caused necrosis of placental tissues. Owen and others (1968) also found generalised coagulative necrosis in the placenta of cattle which aborted after experimental infection with I.B.R virus, and suggested that the uniformity of the lesions was indicative of placental ischaemia caused by gradual impairment of fetal circulation, rather than by viral insult.

Placental lesions associated with L.hardjo infection seemed to be equally non-specific. In about half the placentomes, coagulative necrosis was accompanied by frank haemorrhage together with varying degrees of septal congestion, oedema, and inflammatory cell infiltration. In cases F1387, F1388, F1600, F2811, and F2984 trophoblast cells at the tips of the chorionic villi were degenerate and ballooned, and were associated with hypoxic changes in the fetus. Aborting cattle in these cases all had titres of 1/1600 - 1/2560 to L.hardjo.

The inflammatory response in placentomes with this lesion involved polymorphonuclear rather than mononuclear cells, as in placental lesions found in aborted heifers by Murphy and Jensen (1969), but contrary to the generalised inflammatory response associated with leptospiral infection in man (Lichtenberg 1984). In three cases, there was no

fetal serological response to L.hardjo, and no other aetiological factors were implicated. It may be presumed, therefore, that abortion occurred before the fetus had time to mount an immunological response.

It is likely that an acute fetal septicaemia caused vascular endothelial necrosis within the placentome, leading to hypoxia and fetal death, as well as degenerative changes in trophoblast cells. Since fetal death precedes abortion by several weeks (Ellis 1986), a secondary bacterial infection could have invaded the fetus through a partially-open cervix some days prior to abortion, thereby producing a non-specific bacterial placentitis with a related polymorpho-nuclear inflammatory cell response. In common with Murphy and Jensen (1969), focal haemorrhage, congestion or oedema were also noticed.

On the evidence of maternal blood samples, two cases produced evidence of a concurrent B.V.D infection on a farm, together with a fetal serological response to L.hardjo infection with titres in thoracic fluid of 1/160 and 1/40, respectively. These examples confirm that when there are two or more aetiological factors associated with an abortion, the interpretation of histopathological findings becomes more difficult. For instance, there may be two explanations for the serological results obtained in these cases: chronic placental damage could have caused leakage of maternal immunoglobulins across the feto-maternal barrier into the fetus (Silverstein and Pendergast 1970): on the other hand

there may have been a genuine immunological response to L.hardjo infection by the fetus.

In the two cases mentioned, there was a noticeable absence of haemorrhagic lesions, which poses several questions:-

a) does B.V.D infection, acquired during pregnancy in cattle, compromise normal placental function thereby making it susceptible to secondary bacterial infection by opportunist pathogens, including L.hardjo?

b) if so, does the fetus become less able to mobilise body defences, even to mild secondary bacterial challenge, so that pathological change associated with generalised septicaemia is absent?

c) whilst serological evidence shows that acute L.hardjo infection was present in these cattle at the time of abortion, is this really irrelevant and is B.V.D infection the cause of the placental lesions?

Trophoblast hyperplasia was found in two cases -F1795; F2000 - located at the apex of caruncular septa, not associated with any diagnosis. Epithelium was pseudo-stratified cuboidal, with centrally-placed nuclei. Some vacuolated cells were found within the mass. There was no additional syncytial formation. In human haemochorial placentation, trophoblast hyperplasia has been related to several maternal and placental disease conditions:

i) a reduction in blood supply through the decidua, leading to hypoxia and compensatory stimulation of cyto-

trophoblastic activity (Fox 1970).

ii) damage to trophoblast by hypoxia, which is followed by replacement and repair.

iii) a failure of cytotrophoblast regression, ie., a failure of villous and placental maturation (Fox 1978).

iv) trophoblast proliferation, counteracting any decline in placental hormone synthesis resulting from ischaemia. It is evident from clinical experiance in human medicine that the production of hormones by the placenta either progressively declines or fails to increase with gestation as placental ischaemia becomes more severe. This may lead to a declining quality of trophoblast environment, which could end in abortion (Perrin 1984).

That trophoblast hyperplasia could be a response to abnormal physiological function is not an attractive hypothesis in a species like the cow which has developed an epithelio-chorial placentation. Any increase in the number of cellular layers that interpose themselves between maternal and fetal circulations in the placentome will decrease the efficiency of gaseous exchange, provoking hypoxia rather than compensating for it. With respect to a possible explanation being that of placental immaturity, there is a basic species difference between methods of placental growth in the cow and that in the human. In man, the placenta grows by eruption and lateral spread of cytotrophoblast from the chorionic plate at the apex of primary villi, to form the basal plate of the placenta

through which maternal blood circulates (Steven and Morriss 1975). In the immature placenta, these buds of cytotrophoblast are known as syncitial "knots". This is not a feature of bovine chorionic villous trophoblast; primary villi are simple truncated projections from which secondary and tertiary villi develop, covered by a single layer of syncytio-trophoblast, whose connective tissue core shrinks as pregnancy progresses (Steven and Morriss 1975). Hence, hyperplasia cannot increase the surface area to improve the rate of gaseous diffusion.

A possible explanation could be that hyperplasia is a normal response to intra-cellular trophoblast damage, caused either by infection or through the effects of hypoxia from fetal organic disease. Whilst this hypothesis has been argued for leptospiral infection, the two cases F1795 and F2000 might provide a different slant to the discussion.

This view is based upon other lesions found in these two fetuses:

i) both fetuses exhibited conjunctival hyperplasia and goblet cell formation, together with a sub-conjunctival polymorph cellular infiltration.

ii) there was generalised congestion in fetal lungs, with diffuse interstitial pneumonia involving polymorphonuclear inflammatory cells.

The pneumonic lesion could indicate an infectious aetiology to these cases, spread via the placental route; a focal placentitis was also present. Indeed, the

trophoblastic lesion bears some resemblance to that found in conjunctival epithelium. This is not surprising since the two tissues have a similar embryological origin (Steven 1975: Banks 1981). Any inflammatory reaction within the connective tissue core of the septa was minimal.

The overall picture being proffered is that of a chronic, low-grade disease process which may be compatible with fetal growth or survival. For the fetus to survive to full-term, essential placental functions need to remain fully operative. If the normal endocrine functions of the trophoblast are impaired, hyperplasia may be a specific response to placental dysfunction.

Cotyledonary placenta was the tissue most commonly available for study. It was usually collected with the aborted fetus, or by manual traction on retained fetal membranes per vaginum. Bacterial contamination and tissue necrosis were normally present which limited its diagnostic value. Unlike the maternal caruncle which is composed of an active and viable epithelium, supporting connective tissue, and functional vascular system, the fetal cotyledon has frequently been starved of blood for several days. At the time of collection, degenerative changes have already taken place in this tissue. However, one positive aspect of identifying a leucocyte infiltration within the chorio-allantoic membrane is that the cells found in the villous and membraneous connective tissue stroma are likely to originate from the fetus, and any inflammatory reaction

will not be a continuation of the maternal response.

Varying degrees of post mortem change were seen in most of the cotyledons examined; trophoblast degeneration and necrosis with pyknosis, fibrosis, oedema, vascular sclerosis, and mineralisation, together with some inflammatory cell infiltration. Specific lesions of villitis were useful in assessing the presence of an infection as a possible cause of the abortion. Mineralisation served as an indicator for placental hypoxia, the relevance of which could be gauged from other post mortem changes.

Oedema, accompanied by only a little inflammatory change, was a specific lesion of the inter-cotyledonary chorio-allantois. Occasionally, a low-grade polymorph cell infiltration was noted, with macrophages clustered around terminal branches of the umbilical vessels within the connective tissue stroma. There appeared to be no consistent diagnostic findings associated with the lesion. This lesion is likely to have occurred through the presence of an intact caruncle, with its still-functional blood supply, being in close apposition to non-functional fetal membranes which surround the dead fetus; a progressive, passive chorionic oedema or pseudo-hydropic change could have developed. It is a precursor to calcification and true fibrosis, which are said to occur within about two weeks of fetal death following membrane retention (Altshuler 1984).

The frequency of lesions in chorionic membrane, as distinct from cotyledon, in this study seems to be

significantly lower than that found in human or equine obstetrics. The incidence of human chorio-amnionitis in abortions and stillbirths is around 24% (Fox and Langley 1971) and there is a strong correlation between it and prolonged rupture of the membranes prior to actual birth (Blanc 1961); in mares the incidence is 13% (Whitwell 1980). The comparable figure for this study is 3%. Several conclusions may be drawn from this finding:

i) that infections of the bovine fetus rarely spread from the endometrium through intact inter-cotyledonary chorio-allantoic membrane.

ii) that the intact chorionic membrane is an efficient barrier against most maternal haemat-ogenous infections which are capable of causing abortion.

iii) that the most common route for bovine fetal infections appears to be via the caruncle and trophoblast cell. This observation has been elegantly confirmed by Anderson and others (1986b) in their study on the pathogenisis of placentitis in the goat following experimental B.abortus infection.

v) that infection rarely spreads within the bovine chorio-allantois by lateral, cell-to-cell transfer. The specific infections where this does occur, L.monocytogenes (Racz and others 1972) and B.abortus (Anderson and others 1986a;b), were not encountered in this work.

v) there was no evidence of pathogenic bacteria being shed directly into the uterine lumen and so gaining direct contact with chorionic epithelium at the inter-cotyledonary feto-maternal interface. Since trophoblast is endocytic, infection can enter the chorion in this fashion (Dearden and Ockleford 1983), but the absence of exudative lesions at this site indicates that this is not a feature of the bacterial infections encountered in this study.

Lesions of the amnion.

Amniotic fluid inside intact membranes is usually sterile. When Chany and others (1966) carried out studies with human poliomyelitis virus in monolayer culture of amniotic cells, the intact amnion did not permit growth of the virus in vitro. They suggested that the intercellular matrix of the membrane, rather than the amniotic cells themselves, inhibited the spread of that organism. Galask and others (1974) studied the growth of E.coli in the presence of amniotic fluid by electron microscopy, and found that after culture the medium contained a bacteriostatic factor. The conclusion from this work is that organisms entering the amniotic cavity have a limited ability to colonise the amnion or invade the fetus via skin, upper respiratory tract, or intact mucous membranes.

Chorio-amnionitis is not common in cases of human fetal death, arising most frequently with the induction of labour and the consequently long period between membrane rupture

and delivery of the fetus. In this situation, infection may spread from the inflamed membranes to the fetus causing a congenital pneumonia, meningitis, or neonatal pyogenic dermatitis and ophthalmia. Chorio-amnionitis is viewed as a potential, rather than an actual, threat to the fetus (Fox 1978). Haematogenous spread is not considered a factor in the aetiology of amnionitis.

Focal chronic amnionitis was a feature of 3/10 cases, characterised by fibrosis, mineralisation, and a heavy mononuclear cell infiltration. Two cases - F962 and F1040 - originated from the same farm. They represented the only premature calvings from a group of 14 pedigree Friesian-Holstein heifers in one year. F962 was stillborn and pathological lesions were found in fetal conjunctiva, lung, and placenta. F1040 was born alive and survived; only fetal membranes were examined, and the chorio-allantois was found to be oedematous with a severe haemorrhagic placentitis. The amnion, too, was thickened and oedematous, and multi-focal haemorrhages were present on the fetal surface. F1462 presented lesions similar to those found with case F962. There are no descriptions of similar amniotic and associated fetal lesions elsewhere in the literature, other than an account of lesions in fetal and placental tissues following experimental infection of pregnant heifers with U.diversum (Ruhnke and others 1984). Jones and Tobin (1972) have described the isolation of T-strain mycoplasmas from cases of human placentitis and chorio-amnionitis from material

obtained during Ceasarian section in patients with premature rupture of placental membrane. U.urealyticum was the human strain identified, and it was associated with infants of low birth-weight, chorio-amnionitis, and perinatal death, also involving mycoplasma species (Kundsin and others 1984). It has been concluded elsewhere that mycoplasma infection may well be implicated in the diagnosis of "villitis of unknown aetiology" (VUE) in human medicine (Altshuler and Russell 1975). Altshuler (1984) has observed that even with the resources of human medical diagnostic laboratories, the T-strain mycoplasmas are difficult to culture; they may be more prevalent in autopsy preparations than hitherto realised.

In this study, no consistent bacteriological or serological results were obtained to account for the lesions described. A blood sample taken from calf F1040 at birth produced a high IgG/IgM concentration but no specific titres to other infectious diseases under consideration. Since the chorio-allantoic lesions in all three cases were those of focal necrotic villitis with a heavy mixed inflammatory cell infiltration, it is concluded that that there could be an infectious aetiology to this type of abortion. The lesions recorded here are similar to those described by Ruhnke and others (1984) associated with U.diversum abortions in cattle.

The pathogenicity of U.diversum is not well documented. Certain strains of Ureaplasmas may not be directly patho-

genic for bovine embryos (Britton and others 1987). At this time the various pathological lesions which may be produced by different strains are unknown.

Epithelial metaplasia was a finding in three different sections of amnion examined without other inflammatory lesions being seen. Typically, white, elevated, rough circular areas of up to 2mm diameter were located on the fetal surface of the amnion and extended onto the umbilical cord, where they were more numerous. Bourne (1962) has given a full description of this structure which he noticed in 4% of human placentas. Histologically, these "amniotic plaques" consisted of stratified squamous epithelium covered by a superficial layer of normal keratin. The significance of this finding is uncertain and may not necessarily be an indication of pathological disease. Whilst the normal structure of amniotic epithelial cells can naturally be altered by folds in the membrane itself or tension within the amniotic sac (Hayes 1975), there are suggestions of an link between oligohydramnios, fetal malformations and epithelial metaplasia which Bourne (1962) felt were unsubstantiated. In this study only one case -F1065 - of the three was related to a congenital abnormality, and that was from a B.V.D sero-positive dam.

Oedema and fibrosis were occasionally recorded in this series, of which two cases (F1388; F1400) came from the same farm where an abortion storm was associated with both B.V.D and L.hardjo infections. Another case came from a

different farm where L.hardjo was again implicated in the diagnosis. Since no inflammatory lesions were found it suggests that direct amniotic infection played no part in the lesion's development. Whilst Murphy and Jensen (1969) found severe oedema in the allanto-chorion of heifers infected experimentally with L.pomona, there was little evidence of more widespread histopathological change, and they concluded that membrane oedema was the result of anoxia brought about by the destruction of fetal erythrocytes by leptospiral haemolysins. One further example of this lesion was associated with B.V.D infection alone, again with minimal inflammatory change: it appears that tissue hypoxia from differing causes could produce the lesion described here.

Any difficulties in the interpretation of pathological changes in the placenta of these abortion cases cannot be overcome by comparing the placental lesions which were found in this study to lesions observed after experimental infection with a single infectious agent. Further studies need to be carried out regarding possible inter-relationships between the known abortive agents in cattle and their effects on the placentome, in order to elucidate the pathogenesis of some of these factors in relation to bovine abortion.

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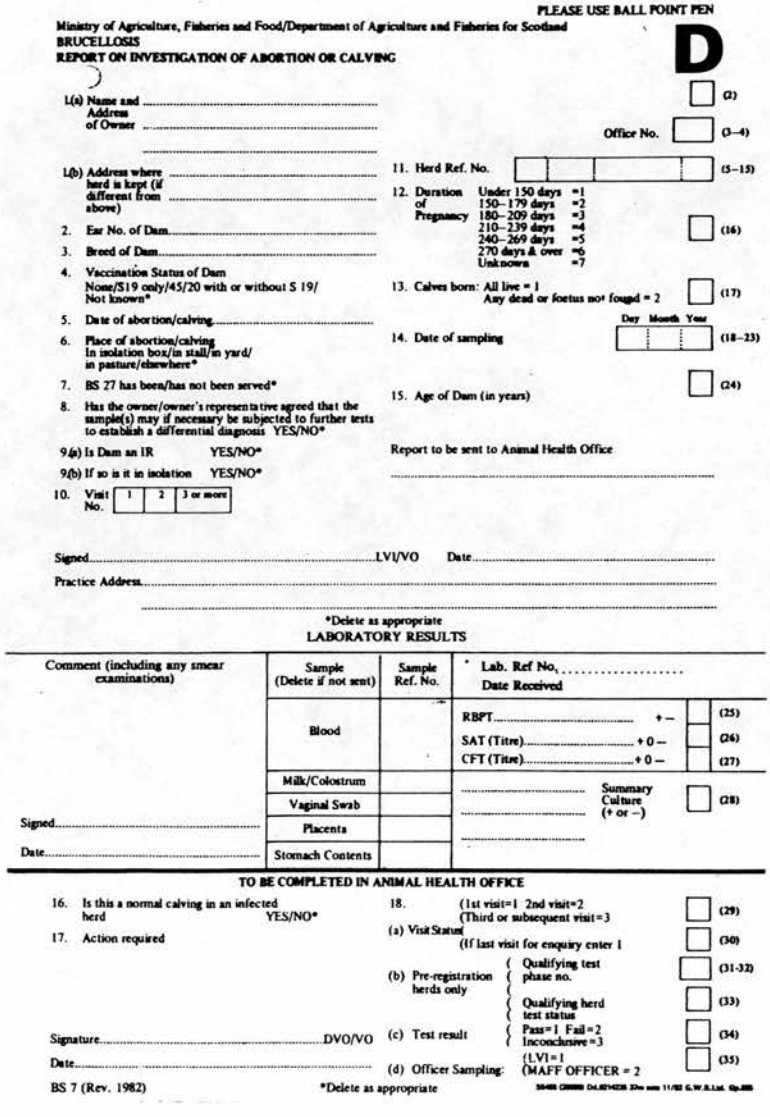
APPENDICES.

Appendix 1.

Ministry of Agriculture, Fisheries and Food/Department of Agriculture and Fisheries for Scotland.

Form BS 7 for the report on investigation of abortion or calving in cattle.



PLEASE USE BALL POINT PEN

Ministry of Agriculture, Fisheries and Food/Department of Agriculture and Fisheries for Scotland

BRUCELLOSIS

REPORT ON INVESTIGATION OF ABORTION OR CALVING

D

(2)

L(a) Name and Address of Owner .....

Office No. (3–4)

L(b) Address where herd is kept (if different from above) .....

11. Herd Ref. No. (5–15)

12. Duration of Pregnancy: Under 150 days =1; 150–179 days =2; 180–209 days =3; 210–239 days =4; 240–269 days =5; 270 days & over =6; Unknown =7 (16)

2. Ear No. of Dam.....

3. Breed of Dam.....

4. Vaccination Status of Dam None/S19 only/45/20 with or without S 19/ Not known*

13. Calves born: All live = 1; Any dead or foetus not fouged = 2 (17)

5. Date of abortion/calving.....

14. Date of sampling — Day Month Year (18–23)

6. Place of abortion/calving In isolation box/in stall/in yard/ in pasture/elsewhere*

15. Age of Dam (in years) (24)

7. BS 27 has been/has not been served*

8. Has the owner/owner's representative agreed that the sample(s) may if necessary be subjected to further tests to establish a differential diagnosis YES/NO*

9(a) Is Dam an IR YES/NO*

9(b) If so is it in isolation YES/NO*

10. Visit No. 1 | 2 | 3 or more

Report to be sent to Animal Health Office .....

Signed.....LVI/VO Date.....

Practice Address.....

*Delete as appropriate

LABORATORY RESULTS

Comment (including any smear examinations)	Sample (Delete if not sent)	Sample Ref. No.	* Lab. Ref No, ... Date Received	
	Blood		RBPT..... + –	(25)
			SAT (Titre)..... + 0 –	(26)
			CFT (Titre)..... + 0 –	(27)
	Milk/Colostrum		..... Summary Culture (+ or –)	(28)
	Vaginal Swab		.....	
Signed.....	Placenta		.....	
Date.....	Stomach Contents			

TO BE COMPLETED IN ANIMAL HEALTH OFFICE

16. Is this a normal calving in an infected herd YES/NO*

17. Action required

18. (1st visit=1 2nd visit=2 (Third or subsequent visit=3 (29)

(a) Visit Status (If last visit for enquiry enter 1 (30)

(b) Pre-registration herds only: Qualifying test phase no. (31-32); Qualifying herd test status (33)

(c) Test result: Pass=1 Fail=2 Inconclusive=3 (34)

(d) Officer Sampling: (LVI=1 (MAFF OFFICER = 2 (35)

Signature.....DVO/VO

Date.....

BS 7 (Rev. 1982) *Delete as appropriate

Appendix 2.

Practice form completed at post mortem examination of aborted fetus.

Breed

Condition of Foetus

Fresh ☐

Autolysed ☐

Mummified ☐

Oedematous ☐

Incomplete ☐

REF

X REF

DATE

Weight ..... kg

Length ..... cms

Sex

Lungs Aerated ☐

Partially Aerated ☐

Not Aerated ☐

	ORGAN	WEIGHT (gms)	
	Whole brain		Into Formalin
	Cerebellum		
	Thyroid		
INTO VTM	Liver) To Virology		
	Lungs) CVL for		
	Spleen) virus isolation	C/Thymus	
	Adrenal		
	Placenta		

TIBIA - X-ray at Leeds

THORACIC FLUID

1. To Virology CVL for virus serology
2. To bacty CVL for Lepto (mark form thoracic fluid).

CULTURES - (cross out any examinations not done.

PLACENTA

BA

MC

F x 2

S CONTENTS

BA

MC

GENERAL COMMENTS

Appendix 3.

Ministry of Agriculture, Fisheries and Food Veterinary Investigation Service reqest form VIO 16.

Ministry of Agriculture, Fisheries and Food
VETERINARY INVESTIGATION SERVICE

ADAS

V.I.Centre Reference .....
Date of receipt .....

1. Name and full farm address of owner .....

Date collected .....

2. Name and full postal address of veterinary surgery (to which report will be sent)

3. For the attention of .....
Previous reference (if applicable) .....

4. Examination required

5. Type of specimen .....

6. Species/Breed .....

7. Identification ..... 8. Age ..... 9. Sex .....

10. State MAFF Health Scheme (if relevant) e.g. Pig/Poultry Health Scheme. .....

11. **Please give case history** e.g. clinical signs/treatment/PM findings etc.

VIO 16 (Rev.1982)

Continued overleaf

FLOCK/HERD DETAILS

12. No. of losses .....
13. Date losses commenced .....
14. No. in group at risk .....
15. No. affected .....
16. Date purchased .....
17. Total stock .....

18. Flock/Herd treatment (including vaccinations/anthelmintics)

19. Housing/Husbandry

20. Feeding

21. Other information

Appendix 4.

Ministry of Agriculture, Fisheries and Food Veterinary Investigation Service report form VIO 16 completed with laboratory results attached.

Case No. F2984. Serological results on fetal thoracic fluid.

Ministry of Agriculture, Fisheries and Food
VETERINARY INVESTIGATION SERVICE

ADAS

V.I.Centre Reference F2984
Date of receipt 25 JUL 1986

1. Name and full farm address of owner: J Lowthe. Tunstall Hall.
Date collected 4/7/86.

2. Name and full postal address of veterinary surgery (to which report will be sent)
The Morn Veterinary Group
The Veterinary Surgery
17 New Road, Kirkby Lonsdale
Carnforth, Lancs. LA6 2AB

3. For the attention of
Previous reference (if applicable)

4. Examination required: IBR
Abortion enquiry -

5. Type of specimen: Foetal fluids.
6. Species/Breed: Bovine, Holstein.
7. Identification: 2200. 8. Age: 9. Sex: ♀

10. State MAFF Health Scheme (if relevant) e.g. Pig/Poultry Health Scheme.

11. Please give case history e.g. clinical signs/treatment/PM findings etc.
PTO.

REPORT from Veterinary Investigation Centre Merrythought, Calthwaite, Penrith Cumbria, CA11 9RR. (Telephone No: Calthwaite (C768 85) 295)

SAMPLE NO	VIROLOGY											
	SEROLOGY					ANTIGEN DETECTION						CELL CULTURE
	BVD SNT.	IBR ELISA	[redacted] Lepto hardjo	[redacted] IgG mg/ml	IgM mg/ml.	BVD FAT	IBR FAT	PIS FAT	RSV FAT			
	—	—	1/40	<1·0	<1·0							

*Examinations are in progress.

D Lovett 10-7-86

A titre of 1/40 to Leptospira hardjo indicates abortion due to this organism.

D Lovett .....D E COUNTER 23.7.86.

Appendix 5.

Ministry of Agriculture, Fisheries and Food Veterinary Investigation Service report form VIO 16 completed with laboratory results attached.

Convalescent blood sample results from two aborted heifers, Case No. F2985.

Ministry of Agriculture, Fisheries and Food
VETERINARY INVESTIGATION SERVICE

ADAS

V.I.Centre Reference F985
Date of receipt 25 JUL 1986

1. Name and full farm address of owner: S Tarbuck, Tunstall Hall
Date collected 4/7/86

2. Name and full postal address of veterinary surgery (to which report will be sent)
The Morn Veterinary Group
The Veterinary Surgery
17 New Road, Kirkby Lonsdale
Carnforth, Lancs. LA6 2AB

3. For the attention of
Previous reference (if applicable)

4. Examination required: Abortion serology x 2 — IBR — BVD — Lepto.

5. Type of specimen: Single sera x 2.

6. Species/Breed: Bovine; Holstein.

7. Identification: 1; 2. 8. Age Heifers 9. Sex F

10. State MAFF Health Scheme (if relevant) e.g. Pig/Poultry Health Scheme.

11. Please give case history e.g. clinical signs/treatment/PM findings etc.
Two abortions:
1) has IBR SN titre of 1/256 (Burton Hall) 2) has IBR SN titre of 1/64

REPORT from Veterinary Investigation Centre Merrythought, Calthwaite, Penrith Cumbria, CA11 9RR. (Telephone No: Calthwaite (0768 85) 295)

SAMPLE NO	VIROLOGY											
	SEROLOGY					ANTIGEN DETECTION						CELL CULTURE
	BVD SNT	IBR ELISA	Lepto hardjo	RSV		BVD FAT	IBR FAT	PI3 FAT	RSV FAT			
1	64	–	1/400									
2	32	–	1/200									

*Examinations are in progress.

Titres to Leptospira hardjo as above. Very high, indicating cause of abortion.

D E COUNTER 23.7.86.

In view of the negative ELISA test we found and your previous findings, we submitted these two samples to Weybridge for SNT for IBR. No titres were detectable by the SN test.

D E COUNTER 28.7.86

Appendix 6.

Ministry of Agriculture, Fisheries and Food Veterinary Investigation Service form VIO 16 completed with results of paired maternal serological examination.

Case No. C327: no fetus was found for examination.

Ministry of Agriculture, Fisheries and Food
VETERINARY INVESTIGATION SERVICE

- 4 MAY 1985

V.I. Centre Reference and date of receipt C 327

1. Name of Owner of animal Mr Wightman
Date Collected 3 15 85
FULL Farm Address Matfen.
NEWBIGGEN.

2. NAME AND FULL POSTAL ADDRESS OF VETERINARY PRACTICE (to which report will be sent.)

MOFFITT, MURRAY & ROBINSON
THE VETERINARY SURGERY
17 New Road, Kirkby Lonsdale
Carnforth, Lancs. LA6 2AB

3. Examination Required
Abortion serology.

V10.16

DETAILS OF ANIMAL

4. Species/Breed Bovine.
(enter Sire first, if mixed crosses enter mixed)
5. Identification (e.g. ear no.) 1) 149 2) A27
6. Age Cows (average if batch)
7. Type of specimen Paired serum. x 2
8. Is herd/flock registered in M.A.F.F. Health Scheme (Pig health, Brucellosis etc
(YES/NO)
9. Sex (tick ONE ONLY as appropriate)

MIXED BATCH	FEMALE						MALE		
	KNOWN PREGNANT				NOT Pregnant	Other	Entire	Cast-rated	OTHER
	First third	Second third	Last third	Stage unknown					
1	2	3	4	5	6	7	8	9	X

REPORT from Veterinary Investigation Centre, Merrythought, Calthwaite, Penrith Cumbria, CA11 9RR. (Telephone No: Calthwaite (0768 85) 295)

SAMPLE NO	VIROLOGY											
	SEROLOGY					ANTIGEN DETECTION						CELL CULTURE
	BVD *	IBR ELISA	PI3	RSV	Lepto *	BVD FAT	IBR FAT	PI3 FAT	RSV FAT			
A29 F	64	0·66			-ve							
A29 S	>128	—			1/100							
149 F	64	—			-ve							
149 S	32	0·93			-ve							

*Examinations are in progress.

SIGNED Mynn DATE 17·5·85

The Leptospira titre above was to L. hardjo.

.....Mynn.....D DYSON 15.5.85.

BVD titres now included above. A29 is being repeated to find an end point titre, since there may be a significant rise.

.....Mynn..... D DYSON 22.5.85

Appendix 7.

Correspondence with Prof.R.B.Miller, Chairman of the Department of Veterinary Pathology at the Ontario Veterinary College, University of Guelph.

Pathological features of Ureaplasma diversum infection in bovine fetuses.

UNIVERSITY OF GUELPH
ONTARIO VETERINARY COLLEGE
Department of Pathology

GUELPH, ONTARIO, CANADA N1G 2W1
Telephone (519) 824-4120

Rerum Cognoscere Causas

August 23, 1985.

Dr. R. D. Murray,
The Morn Veterinary Group,
17, New Road,
Kirkby Lonsdale,
Via Carnforth,
Lancaster, LA6 2AB,
England.

Dear Richard:

I was delighted to receive your letter with all the anecdotes and information regarding your work in the diagnosis of bovine abortion.

We have not published more work on this portion of the ureaplasma project, other than one release for the American Association of Veterinary Laboratory Diagnosticians (photocopy attached). Cornelia's work has been submitted but as yet has not been released. Her work is about infertility rather than abortion and would not seem to be relevant to your inquiry. We will send you a report as soon as the reprints are available.

At this point we believe in order to make a diagnosis of Ureaplasma diversum abortion you must culture the organism from the lung, stomach contents and/or the placenta. In addition to the isolation, there must be a chronic inflammation in the conjunctiva, a primarily nonsuppurative alveolitis preferably with peribronchiolar lymphocytic infiltrations, a chronic fibrosing amnionitis and usually a necrotising chorionitis with mild vasculitis and penetration of the inflammation to the allantoic side. I don't think you can go on isolation alone, but must have the lesions. As far as we know, there aren't a lot of other things that we recognize that will cause a similar lesion. We isolate Ureaplasma diversum from about 14% of the fetuses where we culture for it.

It was very nice to hear from you again and I hope that this information will be helpful to you. Keep up the good work. Very best regards.

Sincerely,

R. B. MILLER, D.V.M.,
Professor and Chairman

RBM:vr
Encl.

Appendix 8.

Summary of the raw data, presented in case order sequence, from 149 abortions investigated between July 1984 and August 1986, and its place within the Results (Table -T- ....; Fig -F- ....) where the case is included.

Case No	C/R length (cm)	Fetal wt (kg)	Material collected						Bacter-iology	Hist-ology	Ser-ology	Included within results at:-
			Fetus	Placen-tome	C.A.M.	Fetal fluid	Maternal serum X2	Other				
F 004	85	23.5	+	–	+	+	+		*	*	*	T 21; 25. F 36; 37.
F 017	82	21.5	+	+	+	+	+		*	*	*	T 16; 17; 18; 20; 24; 26; 27; 28; 29. F 36.
F 028	68	14.5	+	–	+	+	+		*	*	*	T 17; 18; 21; 25; 26. F 36; 37.
F 042	94	30	+	+	+	+	+		*	*	*	T 14; 19; 20; 26; 28; 29.
C 096	33	–	+	–	–	–	+	Mumm	–	–	*	T 21.
F 123	91	25	+	–	+	+	+		*	*	*	T 17; 18; 22; 25; 26; 27. F 37.
C 125	32	–	+	–	–	–	+	Mumm	–	*	*	T 19.
C 130	65	>40	+	–	+	–	+		*	*	*	T 19; 15.
F 160	93	19	+	–	+	+			*	*	*	T 14; 17; 25; 26; 29. F 34.
F 239	90	21	+	–	+	+	+		*	*	*	T 19; 20; 25; 26.
F 240	79	11	+	–	+	+	+		*	*	*	T 16; 17; 19; 20; 29.
F 309	78	19	+	–	+	+	+		*	*	*	T 15; 16; 19; 27.
C 327	–	–	–	–	–	–	+		–	–	*	T 21; 22. F 37.
C 328	13	–	+	–	–	–	+	Mumm	–	–	*	T 19.
F 385	33	1.5	+	–	–	–	+	Twins	–	–	*	T 21.
	32	1.5	+	–	–	–	+		–	–	*	
F 410	35	2	+	–	–	+	+		*	–	*	T 17; 19
F 468	60	7.5	+	–	+	+	+		*	*	*	T 16; 17; 19; 24; 26.
F 548	63	10	+	–	–	+	+	Twins	*	–	*	T 17; 18; 21.
	62	9	+	–	–	+	+		*	–	*	
F 558	66	10	+	+	+	+	+		*	*	*	T 16; 17; 18; 20; 21; 22; 24; 25; 26; 28. F 37.
F 586	21	3	+	–	+	+	+		*	*	*	T 14; 19; 20; 29.
F 588	–	–	–	–	–	–	–	Single blood.	–	–	*	
F 632	59	8	+	+	+	+	+		*	*	*	T 20; 29.
F 712	92	33	+	+	–	+	+		*	*	*	T16;17; 21; 28; 29; F36; 37.
F 750	–	–	–	–	–	–	+		–	–	*	

Case No	C/R Length (cm)	Fetal wt (kg)	Material collected						Bacter-iology	Hist-ology	Ser-ology	Included within results at:-
			Fetus	Placen-tome	C.A.M	Fetal fluid	Maternal serum X2	Other				
C 769	85	–	–	–	–	–	+	Fetus alive	–	–	*	
F 785	63	11	+	+	–	+	+		*	*	*	T 16; 17; 18; 20; 22; 23; 24; 26; 28. F 34; 36; 37.
F 789	76	20	+	+	–	+	+		*	*	*	T14; 20; 29.
F 797	50	6.5	+	–	+	+	+	Twins	*	*	*	T 19; 20; 25; 26; 29; 30. F 36;
	47	5.5	+	–	+	+	+	Amnion	*	*	*	F37.
F 799	62	13	+	+	+	+	+		*	*	*	T 18; 20; 26; 28; 29. F 34.
F 823	94	35	+	–	+	+	+		*	*	*	T 16; 17; 18; 20; 26. F34
C 858	79	–	–	–	–	–	–	Live fetus	–	–	*	
F 886	70	15	+	+	+	+	+		*	*	*	T 15;16; 17; 18; 20; 23; 24; 25; 26; 28; 29. F 36.
C 888	65	10	+	–	+	+	+		*	*	*	T 15; 19; 20.
F 888	66	10	+	+	+	+	+		*	*	*	T 16; 17; 18; 20; 22; 24; 26; 29, F 34; 37.
F 889	75	13	+	+	+	+	+		*	*	*	T 16; 17; 20; 22; 25; 26; 28; 29. F 34; 37.
F 906	19	–	+	–	–	–	+	Mumm	–	–	*	T 21.
F 944	39	3	+	–	–	–	+	Mumm	*	*	*	T 21; 26.
F 961	32	3	+	+	+	–	+		*	*	*	T 14; 20; 21; 28; 29. F 36; 37.
F 962	86	18.5	+	–	+	+	+	Amnion	*	*	*	T 14;16; 17; 19; 20; 24; 26; 27; 29.
F 972	56	7	+	–	+	+	+	Twins	*	*	*	T 14; 19; 20;
	55	7	+	–	+	+	+		*	*	*	21; 26; 29. F 34; 37.
C 981	78	–	–	–	+	–	+	Live	*	–	*	T 20; 22.
C 982	79	–	–	–	–	–	–	fetuses	–	–	*	
F 983	86	19	+	–	+	+	+		*	*	*	T 15; 16; 20; 27; 29; F 34.
F 988	70	13.5	+	+	+	+	+		*	*	*	T 20; 21; 22; 26; 28; 29. F 34; 37.
F 997	66	9	+	+	+	+	+		*	*	*	T 14; 17; 20; 21; 24; 25; 26; 28; 29. F 35.

Case No	C/R length (cm)	Fetal wt (kg)	Material collected						Bacter-iology	Hist-ology	Ser-ology	Included within results at:-
			Fetus	Placen-tome	C.A.M	Fetal fluid	Maternal serum X2	Other				
F 998	–	–	–	–	–	–	+		–	–	*	T 21; 23. F 36.
F1032	76	12	+	+	+	+	+		*	*	*	T 14; 16; 17; 18; 20; 22; 26; 28; 29. F 37.
F1034	88	–	–	–	–	–	+	Live fetus. Blood	–	–	*	T 23.
F1039	83	17	+	–	+	+	+		*	*	*	T 14; 15; 16; 17; 19; 20; 26; 27.
F1040	85	20	–	–	+	–	+	Fetal blood	*	*	*	T 19; 20; 29; F 36; 37.
F1056	40	2.5	+	–	+	–	+	Mascer	*	–	*	T 15; 19; 20.
	35	2	+	–	+	–	+	Twins	*	–	*	
F1065	86	21	+	+	+	+	+	Amnion	*	*	*	T 17; 18; 20;
	88	24	+	+	+	+	+	Twins	*	*	*	21; 26; 30. F 36; 37.
F1103	59	10	+	–	+	–	+	Mascer	*	–	*	T 19; 21; 22; 29. F 37.
F1144	–	–	–	–	+	–	+		–	–	*	T 19; 20; 22; 29. F 37.
F1219	49	5	+	–	–	+	+		*	*	*	T 18; 22; 24; 26. F 37.
F1221	87	23	+	–	+	+	+	Twins	*	*	*	T 16; 19; 20;
	88	24	+	–	+	+	+		*	*	*	22; 24; 25; 26; 29. F 37.
F1224	80	25	+	–	+	+	+		*	*	*	T 15; 16; 17; 18; 20; 21; 24; 25; 26; 27; 29; F 36;37.
F1251	94	28	+	–	–	+	+		*	*	*	T 16; 17; 25; 27.
F1268	–	–	–	–	–	–	+		–	–	*	T 23.
F1276	45	6	+	–	+	+	+		*	–	*	T 14; 19.
F1336	94	32	+	–	+	+	+		*	*	*	T 14; 16; 17; 18; 20; 29.
F1337	67	11	+	+	+	+	+		*	*	*	T 14; 17; 20; 21; 23; 25; 26; 28; 29. F 36.
F1342	–	–	–	–	–	–	+	Mascer	–	–	*	T 22.
C1347	61	11	+	+	+	+	+		*	*	*	T 17; 20; 24; 26; 27; 28; 29. F 34.
F1358	79	27	+	+	+	+	+		*	*	*	T 15; 16; 17; 18; 20; 22; 24; 25; 27; 28; 29; F 37.
F1386	88	–	+	+	+	–	+	Live fetus. Blood	*	*	*	T 19; 20; 28. 29.

Case No	C/R length (cm)	Fetal wt (kg)	Material collected						Bacter-iology	Hist-ology	Ser-ology	Included within results at :-
			Fetus	Placen-tome	C.A.M	Fetal fluid	Maternal serum X2	Other				
F1387	69	15	+	+	+	+	+		*	*	*	T 14; 16; 17; 18; 20; 21; 23; 24; 25; 26; 27; 28. F 36.
F1388	68	14	+	+	+	+	+	Amnion	*	*	*	T 14; 17; 19; 20; 25; 26; 28; 30.
F1389	83	26	+	–	+	+	+		*	*	*	T 14; 16; 17; 18; 20; 21; 23; 24; 27. F36.
F1400	65	12	+	+	+	+	+	Amnion	*	*	*	T 14; 17; 20; 23; 26; 28; 30. F 36.
F1410	86	34	+	+	+	+	+		*	*	*	T 17; 19; 20; 26; 27; 28; 29. F 36.
F1438	36	5	+	–	+	+	+		*	*	*	T 16; 20; 21; 23; 24; 25; 26; 27. F 37.
F1439	–	–	–	–	–	–	+		–	–	*	T 22.
F1444	50	6	+	–	–	+	+		*	–	*	T 19.
F1452	50	5	+	–	–	+	+		*	*	*	T 16; 21; 25; 26; 27; F34; 36.
F1462	78	13	+	–	+	+	+	Amnion	*	*	*	T 15; 16; 20; 21; 24; 25; 26; 27; 29; 30. F36; 37.
F1472	46	4.5	–	–	–	–	+		–	–	*	T 21; 22. F 37.
F1492	90	35	+	–	–	+	+		*	*	*	T 19; 18; 24; 26.
F1505	68	14	+	–	–	+	+		*	*	*	T 16; 21; 22; 24; 26. F 37.
F1522	32	3	–	–	–	–	+	Mascer	–	–	*	T 19; 21; 22.
F1556	80	24	+	–	–	+	+		*	*	*	T 16; 21; 22; 27. F 37.
F1580	81	20	+	–	–	+	+		*	*	*	T22; 18; 17; 16; 24; 26; 27; F 34; 37.
F1581	58	7	–	–	–	+	+	Mascer	*	–	*	T 21.
F1590	81	23	+	+	–	+	+	Twins	*	–	*	T 15; 17; 18;
	83	25	+	+	–	+	+		*	–	*	20; 21. F36
F1599	92	37	+	–	–	+	+		*	*	*	T 16; 17; 21; 24; 25; 26; F 36; 37.
F1600	56	5	+	–	+	+	+		*	*	*	T 17; 20; 23; 26; 29. F 36.
F1607	–	–	–	–	–	–	+	Mascer	–	–	*	
F1624	77	13	+	+	+	+	+		*	*	*	T 16; 17; 19; 20; 26; 27; 28; 29.
F1639	62	13	+	–	–	+	+		*	*	*	T 16; 17; 18; 21.26. F 36; 37.

Case No	C/R length (cm)	Fetal wt (kg)	Material collected						Bacter-iology	Hist-ology	Ser-ology	Included within results at:-
			Fetus	Placen-tome	C.A.M	Fetal fluid	Maternal serum X2	Other				
F1691	–	–	–	–	–	–	+		–	–	*	
F1720	46	4.5	+	–	+	+	+	Amnion	*	*	*	T 14; 17; 19; 20; 24; 26; 29.
F1721	90	27	+	–	–	+	+		*	*	*	T 16; 17; 18; 19; 24; 27.
F1726	90	25	+	–	+	+	+		*	*	*	T 16; 19.
F1742	32	3	–	–	–	–	+	Mumm	–	–	*	T 21; 22.
F1780	40	5	+	–	–	+	+		*	–	*	T 21.
F1795	75	15	+	+	+	+	+		*	*	*	T 16; 17; 18; 20; 24; 26; 27 28; 29. F34.
F1796	84	18	+	+	–	–	+		*	*	*	T 19; 20; 28.
F1799	70	27	+	–	–	–	+	Mascer	*	–	*	T 19.
F1913	59	4.5	+	–	–	–	+		*	–	*	T 17; 19.
F1949	80	24	+	–	–	+	+		*	–	*	T 16; 17; 19.
C1958	–	–	–	–	–	–	+		–	–	*	T 22.
F1981	56	6	+	+	+	+	+		*	*	*	T 16; 17; 18; 20; 24; 26; 27 28; 29. F34.
F2000	84	21	+	+	+	+	+		*	*	*	T 16; 19; 24; 26; 27; 28.
F2026	62	9	+	–	_	–	+	Mascer	–	*	*	T 16; 17; 22; 23.
F2091	–	–	–	–	+	–	+		–	*	*	T 19.
F2108	75	17.5	+	–	–	+	+		*	–	*	T 15; 16; 17; 18.
F2138	62	9	+	–	–	+	+		*	–	*	T 16; 17; 23.
F2139	61	10	+	–	–	+	+		*	–	*	T 16; 19.
F2174	–	–	–	–	–	–	+		–	–	*	
F2175	49	3	+	–	–	+	+		*	–	*	T 17; 19.
F2351	60	13	+	–	+	+	+		*	*	*	T 16; 17; 19; 20; 29.
F2394	56	13	+	–	–	+	+		*	*	*	T 15; 16; 17; 18; 26; 27.
F2526	64	15	+	–	+	+	+		*	*	*	T 17; 20; 24; 27. F 34.
F2589	55	7	+	–	+	+	+		*	*	*	T 16; 17; 18; 20; 24; 26; 28 29. F 34.
F2591	76	22	+	+	+	+	+	Amnion	*	*	*	T 16; 17; 18; 20; 21; 24; 26 28; 30. F 36; 37.
F2652	47	3	+	+	–	+	+		*	*	*	T 17; 19; 20; 28.
F2729	85	24	+	–	+	+	+	Amnion	*	*	*	T 18; 20; 27; 28; 29;30. F 34.
F2731	90	23	+	–	–	+	+		*	*	*	T 17; 22; 23; 26. F 34; 36; 37.

Case No	C/R length (cm)	Fetal wt (kg)	Material collected						Bacter-iology	Hist-ology	Ser-ology	Included within results at:-
			Fetus	Placen-tome	C.A.M	Fetal fluid	Maternal serum X2	Other				
F2733	68	15	+	–	+	+	+		*	*	*	T 16; 17; 18; 19; 26; 29;
F2756	24	1	–	–	+	–	+	Mascer	–	–	*	T 23.
F2811	46	4	+	–	+	+	+		*	*	*	T 16; 17; 18; 20; 21; 23; 26; 27; 28; 29. F 34; 36.
F2849	–	–	–	–	–	–	+		–	–	*	T 21.
F2879	79	16	+	–	+	+	+		*	*	*	T 15; 17; 20; 24; 26. F 34.
F2915	68	7	+	–	+	+	+		*	*	*	T 16; 17; 19; 20; 24; 27; 29.
F2916	–	–	–	–	–	–	+		–	–	*	T 21.
F2917	80	13	+	–	+	+	+		*	*	*	T 19; 20; 26; 29.
F2961	40	2.5	+	+	+	+	+		*	*	*	T 17.
F2984	74	14	+	+	+	+	+		*	*	*	T 17; 18; 20; 21; 23; 25; 26; 28; 29. F 36.
F3023	58	5	+	–	+	+	+		*	*	*	T 20; 24; 26; 29. F 34.
F3034	22	1	–	–	–	–	+	Mumm	–	–	*	
F3045	–	–	–	+	+	–	+	Mascer	–	*	*	T 19; 20; 28; 29.
F3048	20	0.5	+	–	–	–	+	Mumm	–	–	*	T 23.
F3049	–	–	–	+	+	–	+		–	*	*	T 19; 20; 28; 29.
F3050	67	13	–	–	–	–	+		–	–	*	T 21.
F3060	84	19	+	+	+	+	+		*	*	*	T 16; 17; 20; 23; 26; 27; 28; 29; F36.
F3074	52	9	+	–	+	+	+		*	*	*	T 17; 20; 23; 26; 29. F 36.
F3078	46	3	+	–	+	+	+		*	*	*	T 17; 19; 20; 24; 26; 29.
F3148	–	–	–	–	–	–	+		–	–	*	T 22.
F3174	–	–	–	–	–	–	+		–	–	*	T 21.
F3180	62	10	+	–	+	+	+		*	–	*	T 14; 16; 17; 19; 20.
F3223	88	24	+	+	+	+	+	Amnion	*	*	*	T 16; 17; 18; 20; 23; 26; 27; 28; 30. F 36.
F3224	80	20	+	–	+	+	+		*	–	*	T 14; 19; 20.
F3318	52	4.5	+	–	+	+	+		*	–	*	T 14; 19; 20.
F3328	–	–	–	–	–	–	+		–	–	*	T 21; 23.
F3339	84	17	+	–	+	+	+		*	–	*	T 14; 17; 19; 20.
F3354	–	–	–	–	–	–	+		–	–	*	T 21.
F3384	–	–	–	–	–	–	+		–	–	*	T 21.
F3413	71	10	+	–	–	–	+	Mascer	*	–	*	T 15; 19.
F3461	83	20	+	–	–	+	+		*	–	*	T 16; 17; 18; 19.

Key to Appendix 8.

–	Specimen not available or laboratory procedure not carried out.
+	Specimen collected.
*	Laboratory procedure completed.
Mumm	Fetus mummified.
Mascer	Fetus mascerated.
Amnion	Amnion collected at time of fetal delivery.
Fetal blood	Live fetus born; fetal blood sample taken.
Twins	Twins born to one dam.

The following key allocates individual cases to their correct position within the results tables:

T 14	Fetal and placental oedema related to autolytic changes or hepatomegaly in 21 cases.
T 15	Gross abnormal external features in 15 cases and their fetuses.
T 16	Gross pathological findings in the abdominal cavities of 51 fetuses; several features may be shared. F34 related to the case indicates 20 fetuses where fetal septicaemia was associated with the lesions.
T 17	Gross pathological findings in thoracic cavities of 65 fetuses. Many cases exhibited microscopic lesions in the heart (see under T25) and lungs (see under T26).
T 18	Various gross pathological findings in 34 fetuses which exhibited cardiac lesions, with suggested diagnoses (T 21 indicates B.V.D; T 22 indicates I.B.R; T 23 indicates *Leptospira hardjo*; F 34 indicates bacterial or fungal association).
T 19	Gross lesions in 48 fetuses where no diagnostic associations were made.
T 20	Gross placental lesions found in 66 abortion episodes.
T 21	Four-fold change in titres to B.V.D virus infection in 39 cases.
T 22	Four-fold changes in titres to I.B.R virus infection in 21 cases.
T 23	Sero-positive titres at >1/400 to *L.hardjo* in 16 cases.
T 24	Histological lesions in fetal eyelid.
T 25	Histological lesions in fetal heart.
T 26	Histological lesions in fetal lung.
T 27	Histological lesions in fetal liver.
T 28	Histological lesions in placentome.
T 29	Histological lesions in chorio-allantoic membranes,
T 30	Histological lesions in amnion.
F 34	Bacterial or fungal isolates associated with histological lesions in specific tissues from 20 cases; cross reference with T 24 - 30.
F 36	Sero-conversion to B.V.D and/or *L.hardjo* associated with histological lesions in specific tissues from 37 cases; cross reference with T 21, 22, and T 24 - 30.
F 37	Sero-conversion to B.V.D and/or I.B.R associated with histological lesions in specific tissues from 40 cases; cross reference with T 21, 22, and T 24 - 30.