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The diagnosis of salmonella abortion in cattle with particular reference to *Salmonella dublin*. A review

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SUMMARY

The diagnosis of abortion in cattle caused by *Salmonella dublin* depends upon the isolation of the organism from either the products of conception, uterine discharges, vaginal mucus or milk together with serological evidence of active infection. *S. dublin* may be isolated when an active or a latent carrier cow aborts but in these cases an active infection will not be demonstrable. The retrospective identification of a case of *S. dublin* abortion may prove difficult as excretion of the organism is usually transient and the serum agglutinating antibodies frequently fall to low titres soon after the abortion.

INTRODUCTION

The salmonellas, though typically enteric pathogens, are also a well recognized cause of abortion in cattle with the 'host-specific' serotype *Salmonella dublin* being the most commonly isolated (Hinton, 1971). In 1973 salmonellas were numerically more important than *Brucella abortus* as a cause of abortion in Great Britain (Report, 1975) and this trend will probably continue as brucella infection is eradicated from the national herd.

The majority of cases of *S. dublin* abortion occur without other clinical signs (Hinton, 1974) though pregnant cows with *S. dublin* dysentery frequently abort (Field, 1948). The majority of abortions occur sporadically in the summer and autumn with only one or two cases in an outbreak, though on occasions there may be six or more cases. There does not appear to be any specific age or breed incidence though most cases occur in the second half of pregnancy (Hinton, 1971).

This review consists of two main sections. The first part deals with the general aspects of the isolation of *S. dublin* while the second part is concerned with the bacteriological and serological examination of specimens from abortion cases.

PART I. ISOLATION OF *SALMONELLA DUBLIN*

A general review on the isolation of *Salmonella* has been provided by Harvey & Price (1974). Many media have been assessed both experimentally and for diagnostic purposes with much of the work being directed towards identifying material infected with very small numbers of organisms, e.g. animal feeding stuffs, bulk milk supplies, faeces from carriers, food, sewage and water. The most laborious

combination of procedures is unlikely to achieve all possible isolations (Jameson, 1962) but as it can be expected that in clinical disease the organisms will be excreted in relatively large numbers a simple routine will probably be sufficient for diagnostic purposes.

Enrichment techniques

The literature indicates that in England and Wales selenite F broth (SFB) is frequently used as an enrichment medium for the isolation of *S. dublin*. Harvey & Price (1975) found that it was statistically more efficient than brilliant green selenite broth (BGSB), Muller–Kauffmann tetrathionate broth (TB) and Rappaport's magnesium chloride malachite green broth (RB). McCaughey, McClelland & Hanna (1971) also recorded that RB was less efficient than SFB though Smith (1952) found that though SFB was slightly more effective than a tetrathionate broth without brilliant green, there was a definite advantage in using the two in parallel.

The literature regarding TB and BGSB is contradictory. In an experimental study SFB and TB proved less inhibitory to *S. dublin* than BGSB (Kossakowska & Kafel, 1972) but, on the other hand Frik (1969) concluded that BGSB was superior to TB.

Incubating SFB at 43 °C rather than 37 °C appears to be more effective for the isolation of *Salmonella* from heavily contaminated samples, e.g. sewage and abattoir drain swabs, though there was no particular advantage in using the higher temperature for *S. dublin* (Harvey & Price, 1975). There is less contamination of the plating medium with other organisms when the higher temperature is used (Harvey & Thomson, 1953) and the reason for this appears to be that these organisms are being inhibited rather than the *Salmonella* being specifically favoured (Carlson & Snoeyenbos, 1972).

Smith (1952) found that no salmonellas were detected before 15 h incubation in SFB while the optimum time to subculture was 24–30 h. Many investigators subculture twice at 24 and 48 h and in view of the observations by Chattopadhyay & Pilfold (1976) it would seem that this routine should identify most of the active infections.

Selective plating media

Deoxycholate citrate agar (DCA), frequently Hynes' modification, is commonly used for the isolation of *S. dublin* and it is specifically recommended by Harvey & Price (1974). It is frequently used in combination with other selective media and the advantage of this has been illustrated by Le Guilloux (1970), Magee & Hinton (1974) and Harvey & Price (1975).

Harvey & Price (1975) prefer to use DCA in parallel with MacConkey medium containing brilliant green (BGM) for the isolation of *S. dublin* even though some strains may be inhibited by the dye (Harvey & Price, 1968). Certainly the colony size of *S. dublin* is smaller on BGM than on DCA or salmonella shigella agar (SSA) (Harvey & Price, 1975) while experimentally Read & Reyes (1968) found that *S. dublin* was the most sensitive of five salmonella serotypes to brilliant green. Nevertheless, Magee & Hinton (1974) found that BGM with 1/25000 brilliant green

Table 1. *The isolation of* Salmonella dublin *from samples of bovine faeces on deoxycholate citrate agar and brilliant green phenol red agar*

	No. of samples yielding *S. dublin*		
	DCA	BGPR	Total
Direct culture	23	26	27
After SFB enrichment	24	28	28
Total	47	54	55

was as effective as DCA in the isolation of *S. dublin* from bovine diagnostic material.

Frik (1969) reported favourably on the use of brilliant green phenol red agar (BGPR) and a small trial undertaken by the author which compared BGPR and DCA for the isolation of *S. dublin* from bovine faeces indicated that BGPR was slightly superior (Table 1).

Le Guilloux (1970) found that SSA and DCA were equally efficient in the isolation of the serotype from fetal membranes, and Harvey & Price (1967, 1968) indicate that DCA and SSA together are useful for the isolation of *S. dublin* though they suggest that the SSA should be incubated at 40 °C.

Bismuth sulphite agar appears toxic to *S. dublin* and as it is less effective than either DCA or BGM (Harvey & Price, 1975) its use cannot be recommended.

A MacConkey based medium recently described by Shanson (1975) has yet to be evaluated for *S. dublin* though preliminary results indicate that it is similar in efficiency to DCA but with the advantage of being more inhibitory to the non-pathogenic non-lactose fermenters (Davies, 1975).

Combinations of media

A popularly used combination is SFB and DCA while SFB and BGM also appears satisfactory (Magee & Hinton, 1974; Harvey & Price, 1975). Frik (1969) recommends the use of BGSB and BGPR together although the findings of Kossakowska & Kafel (1972), Richardson & Fawcett (1973) and Harvey & Price (1975) suggest that this combination may be relatively inhibitory to *S. dublin* and should not be used. On the other hand, Findlay (1971) noted that in examining slurry the BGSB/BGPR combination was more effective than SFB/DCA though part of the reason for this may have been because the SFB was incubated at 37 °C and not 43 °C.

Richardson & Fawcett (1973) made the interesting observation that though BGSB/BGPR and SFB/DCA were equally satisfactory under experimental conditions the first combination was significantly less efficient for diagnostic samples because one of the four biotypes (Walton, 1972) isolated appeared particularly sensitive to brilliant green.

Preparation of media

It is not the purpose of this review to discuss medium preparation in detail although obviously this must be one of the reasons why differences in the salmonella isolation rate are observed between laboratories.

Many laboratories will use commercially available media though Harvey & Price (1974) find that laboratory-prepared medium is often more efficient. It is appropriate to mention that these authors recommend that SFB is sterilized by filtration rather than by heat while new batches of brilliant green for use in BGM should be titrated against test organisms so that the optimum concentration of the dye can be calculated.

Some properties of Salmonella dublin

S. dublin is an aerobic gram negative bacillus with the antigenic formula 'O' 1, 9, 12 'H' gp. Some strains possess the Vi antigen (Le Minor & Nicolle, 1964).

A number of properties of *S. dublin* including phage typing (Smith, 1951), biotyping (Hall & Taylor, 1970; Walton, 1972; Hinton, 1972*b*) and bacteriocinogenicity and bacteriocin sensitivity (Wray & Clark, 1974) have been investigated, but in general the results have not helped in the study of either the epidemiology or the pathogenesis of the infection.

About a fifth of strains fail to produce gas in glucose (Walton, 1972; Hinton, 1972*b*). The anaerogenic strains are less virulent for mice (Walton, 1972) though there is no difference in the distribution of these strains isolated from adult cattle with dysentery or abortion, or from calves (Hinton, 1972*b*).

Dissociation of *S. dublin in vivo* has been demonstrated by Thal & Holmquist (1957) and Smith (1965) developed a live vaccine from a part rough strain designated HWS 51. Attempts to distinguish the vaccine strain from part rough field strains have so far been unsuccessful (Davies & Sojka, 1971; Walton & Hadfield, 1975).

The *in vitro* antibiotic sensitivity of *S. dublin* strains isolated from abortions, adult cattle with enteritis and from calves were compared using two different techniques but no significant differences in the sensitivity patterns were demonstrated (Hinton & Williams, in preparation).

Recommended procedure for the isolation of S. dublin

The media of choice for the isolation of *S. dublin* include SFB for enrichment and DCA, BGM for the solid plating media with BGPR and SSA as additional possibilities. RB and bismuth sulphite agar appear to be too inhibitory for use with this serotype. Subculturing from SFB at 24 and 48 h should identify most cases of active infection while incubation of the SFB at 43 °C appears to be advantageous in that it reduces the numbers of non-pathogenic non-lactose fermenters isolated.

PART II. EXAMINATION OF SPECIMENS FROM ABORTION CASES

A wide range of bacteria, fungi and viruses have been associated with abortion in cattle including *Br. abortus*, *Campylobacter fetus*, *Corynebacterium pyogenes*, *Leptospira* spp., *Listeria monocytogenes*, *Salmonella* spp., *Aspergillus fumigatus*, *Mucor* spp., bovine rhinotracheitis virus and mucosal disease virus. Each one of these pathogens presents diagnostic problems and consequently a fully comprehensive diagnostic enquiry is not likely to be practicable and manageable if large numbers of abortions are to be investigated. As a consequence any diagnostic routine will represent some measure of compromise and indeed in England and Wales, when salmonella abortion was relatively uncommon, the Veterinary Investigation Centres of the M.A.F.F. frequently used to rely on MacConkey medium for the differentiation of the lactose and non-lactose fermenting Enterobacteriaceae (Hinton, 1972*b*).

It will subsequently be shown that when salmonella infections are specifically suspected as a cause of bovine abortion the use of both selective enrichment broths and salmonella media are to be recommended while serological tests prove a useful adjunct in making a diagnosis.

Microscopical examination

In the diagnosis of *Brucella abortus*, smears of the cotyledon or fetal stomach contents may be stained with either Koster's stain or the modified Ziel–Nielson technique as a screening procedure while a more specific fluorescent antibody test (FAT) has also been described (Corbel, 1973). There are no obvious stains suitable for specifically detecting *Salmonella* in smears of the cotyledon or fetal stomach contents and though the FAT has been adapted to detect salmonellas its use in the diagnosis of salmonella abortion has yet to be evaluated.

Bacteriological examination

S. dublin can be isolated, often in pure culture, from a variety of specimens including the fetus, fetal membranes, uterine discharge, vaginal mucus and milk. *S. dublin* is frequently excreted in the faeces after abortion (Hinton, 1974) so reference to its examination will be made in this section, although normally a faeces sample would not be included with the specimens submitted from a case of abortion.

It is important that when specimens are examined for *Salmonella* selective salmonella media are used and not MacConkey agar. This point was illustrated by Hynes (1942) and also by Hinton (1972*b*) whose results, which are listed in Table 2, show that BGM and DCA were superior to MA for the isolation of *S. dublin* from the fetal membranes.

Fetal stomach and fetal membrane

S. dublin was isolated on direct culture on BGM and DCA from most cases in which there was serological evidence of active infection while most cases in which there was no active infection were only identified after enrichment in SFB (Table 2) (Hinton, 1972*b*).

Table 2. *The isolation of* Salmonella dublin *from the fetal membranes and fetus*

	Material examined			Fetal membrane				Fetal stomach				Total			
	Fetal membrane	Fetal stomach	Both specimens	MA	BGM	DCA	SFB	MA	BGM	DCA	SFB	MA	BGM	DCA	SFB
Active infection (Groups I, II and III)*	10	2	3	7/13	11/13	11/13	13/13	5/5	5/5	4/5	5/5	12/18	16/18	15/18	18/18
No active infection (Group IV)*	5	0	1	0/6	2/6	2/6	5/6	0/1	0/1	0/1	0/1	0/7	2/7	2/7	6/7
Total	15	2	4	7/19	13/19	13/19	18/19	5/6	5/6	4/6	6/6	12/25	18/25	17/25	24/25

* See Hinton (1974).

Table 3. *A comparison of the isolation of* Salmonella dublin *and* Brucella abortus *from fetal membranes and vaginal swabs*

	Fetal membrane	Vaginal swab
No. examined	1877	190
S. dublin positive	106 (5·6)	4 (2·1)
Br. abortus positive	282 (15·0)	27 (14·2)

Figures in parentheses are percentages.

Vaginal mucus samples

Only a few swabs of vaginal mucus were included in the initial samples of the author's 111 cases so that it is not possible to determine the value of the swab for diagnostic purposes from those data (Hinton, 1972*b*). However, the results from over 2000 abortion investigations in which MA was used to screen for *Salmonella*, and serum dextrose antibiotic agar for the isolation of *Br. abortus* (incubated for 4 days at 37 °C in an atmosphere containing 10 % CO_2) have been analysed and the results are listed in Table 3. The isolation rate of *S. dublin* was significantly lower ($P = < 0{\cdot}05$) from the vaginal swabs than from the fetal membranes although there was little difference in the case of *Br. abortus*. This finding is difficult to explain as most of the swabs received were made of alginate and *S. dublin* survives for at least a week on that material (Harrison, 1964). However, it is possible that the MA was not sufficiently selective and better results would have been obtained with salmonella media especially when used in conjunction with SFB enrichment.

Milk

If a milk sample can be obtained at the time of abortion *S. dublin* can frequently be isolated though excretion does not usually persist beyond 4 weeks (Hinton, 1973*b*). The author's technique was to incubate equal quantities of milk and double strength SFB, though the culturing of the cream layer directly on selective media may prove a useful alternative.

When *S. dublin* was isolated from milk the whey H titre was 80 or more though such titres may be found in samples which were negative on culture (Hinton, 1973*b*).

Faeces

S. dublin can be regularly isolated from the faeces of cows after abortion though excretion is usually transient and does not persist for more than a few weeks (Hinton, 1974).

The literature as to whether faecal swabs are preferable to faeces samples is confused. McCall, Martin & Boring (1966) consider that swabs are inefficient if there are less than 1000 organisms/g of faeces, but Richardson & Fawcett (1973) found that a swab would detect *S. dublin* after enrichment in SFB if the sample contained as few as 100 organisms/g, though with direct culture on DCA detection was possible only if the sample contained 10000 organisms/g. Sojka, Thomson & Hudson (1974) indicated that faeces samples would probably be better than swabs

Table 4. *The interpretation of the* Salmonella dublin *serum agglutination test and the whey agglutination test*

	Somatic O titres			Flagellar H titres		
	Negative	Doubtful	Positive	Negative	Doubtful	Positive
SAT	⩽ 20	40	⩾ 80	⩽ 40	80–160	⩾ 320
WAT	Not applicable			⩽ 10	20–40	⩾ 80

for identifying faecal excretion, but on the other hand Frik (1969) considers that swabs are as efficient as faeces in the identification of carriers.

Mixed infections

Br. abortus and *S. dublin* infection may occur together either in the same animal or in the same herd outbreak (Report, 1967; Le Guilloux, 1970; Maclaren, 1972; Hinton, 1974). In addition *A. fumigatus* and *C. pyogenes* infections may co-exist with *S. dublin* and serological examination will assist in determining which is the active infection (Hinton, 1974).

Other serotypes

This review has primarily been concerned with the diagnosis of *S. dublin* abortion. Nevertheless other serotypes may be associated with the condition and at least 28 others including *S. typhimurium* and *S. paratyphi B* have been recorded. Hinton (1971) referred to 18 and to these the following can be added: *S. anatum*, *S. coeln*, *S. hadar*, *S. havana*, *S. indiana*, *S. java*, *S. saintpaul* and *S. thompson* (Le Pennec, 1970; Hinton, 1972*b*; Report, 1972; V.I. Service, 1975).

Serological tests

Serological techniques have been used to assist in the diagnosis of salmonella abortion by several workers including Le Guilloux (1968–75), Cottereau, Rancien & Sendral (1970) and Hinton (1973*a*, *b*). Le Guilloux's approach was to develop techniques for testing a single blood sample collected within 48 h of abortion while Hinton (1973*a*, *b*) concentrated on the examination of paired samples of both serum and whey.

The serum agglutination test

In Great Britain the majority of serological investigations into bovine salmonellosis have used the serum agglutination test (SAT) with both the somatic O and the flagellar H agglutinins being measured. At present there is no standardization of either technique or antigen production but it is possible to suggest a guide for interpretation based on work done in this country (Hinton, 1973*a*). This is summarized in Table 4. Le Guilloux (1975) who used different methods, quotes slightly different values for the SAT. He considers an O titre of 100 and an H titre of 200 as positive but makes no provision for a doubtful category.

One of the drawbacks to the SAT is the presence of non-specific agglutinins in the sera of normal cattle and this explains why only relatively high dilutions are

considered positive. Le Guilloux (1969, 1970, 1971, 1975) found that it was possible to reduce the problem of non-specificity when measuring the O antibodies by increasing the density of the antigen from 5×10^8 to $2{\cdot}5 \times 10^9$ organisms/ml, by incubating the test at 65 °C and by using 0·5 % phenol saline as the dilutent.

Hinton (1973*a*) found that the SAT was a reasonably specific test especially when paired samples are examined within one month of the abortion, and recommends that, though the H titre is marginally superior to the O titre for diagnosis, both titres should always be determined. Conversely Le Guilloux (1975) indicates that the O titre is more valuable than the H and that it is similar in efficiency to the complement fixation test (CFT). Nevertheless, he recommends that at least two of the three tests (SAT, O and H and CFT) should be performed in order to maximize efficiency.

It is not always possible to detect active infection in abortion cases from which *S. dublin* is isolated (Hinton, 1973*a*) and the significance of this in relation to the carrier animal will be considered in a later section.

Haemolytic tests

Le Guilloux (1971, 1972*a*, 1975) developed a complement fixation test (CFT) and also modified Debain's rapid haemolytic test for syphilis in an attempt to overcome the problem of non-specificity. Good agreement was obtained between these tests within 48 h of abortion and neither appear to be affected by a non-specific reaction. In an epidemiological study of *S. dublin* infection Lawson, McPherson & Wooding (1974) reported favourably on a CFT. They used both a phenolized and alcohol treated somatic antigen but gave no data on the results obtained in individually affected animals.

On the other hand Wray & Sojka (1976) concluded that though the CFT provides a guide for recent *S. dublin* infections, including experimental *S. dublin* abortion, the test lacks specificity.

Indirect haemagglutination test

Wray, Morris & Sojka (1975) investigated the indirect haemagglutination test (IHA) but found that there was no advantage in using it instead of the SAT for O and H agglutinins especially in view of the technical problems associated with standardization of the IHA antigens.

S. pullorum *agglutination test*

Le Guilloux (1975) used a stained *S. pullorum* antigen as a screening test and found that 104 of 156 sera from abortion cases caused agglutination within 20 sec and all but 16 were positive within 1 min. He concluded that the test may have an application for screening sera but the results should be confirmed using more specific serological tests.

Whey agglutination test

The flagellar agglutinins can be measured in whey and this test can provide a useful alternative to the SAT especially if paired samples are examined (Hinton,

Table 5. *The distribution of* Salmonella dublin *flagellar agglutinins in vaginal mucus and serum*

Serum flagellar agglutination titres	Vaginal mucus flagellar agglutination titres						
	⩽ 10	20–40	80–160	320–640	1280–2560	⩾ 5120	Total
80–160	1	—	1	—	—	—	2
320–640	—	1	1	1	2	—	5
1280–2560	1	3	2	—	1	—	7
2560	—	1	1	2	1	1	6
Total	2	5	5	3	4	1	20

1973*b*). A guide for the interpretation of the test is listed in Table 4. The salmonella agglutinins appear susceptible to fat solvents and consequently these should not be used for defatting the milk (Hinton, 1972*a*).

Milk ring test

The milk ring test (MRT) has been used extensively as a screening test in the diagnosis of brucella infections. A small trial using a formalized *S. dublin* antigen stained with Harris haematoxylin and suspended in 0·5% phenol saline proved disappointing as many samples with a positive MRT had whey H titres of ⩽ 20 while three samples with titres of 640 or more were negative to the MRT (Hinton, 1973*b*).

Vaginal mucus agglutination test

McCaughey & Hanna (1969) recorded the presence of salmonella agglutinins in the vaginal mucus of a heifer that had aborted. Hinton (1972*b*) examined 20 mucus samples from 14 cases of abortion. The mucus was diluted 1/5 in physiological saline, emulsified and then centrifuged before testing. It was only practicable to test for H agglutinins and these results are listed together with the serum titres in Table 5. Titres of 20 or more were detected in 18 of the 20 mucus samples but there was no obvious correlation between the serum and mucus titres.

There are no established diagnostic criteria for this test and it is therefore probably best interpreted together with the other tests.

Fetal pathology

A small number of fetuses and fetal membranes have been examined by several workers but the results obtained suggest that there are no specific pathological changes on which to make a diagnosis (Bert, 1943; Bishop, Schatz & Canham, 1943; Avery & Niilo, 1963; Dennis, 1969; Hinton, 1972*b*; Plinguier, 1974).

Abortion in carrier cows

Richardson (1973*b*) made the interesting observation that the incidence of dystokia, still births and retention of the placenta was higher in cows which are latently affected with *S. dublin* than in normal cattle. Richardson (1973*a*) has also shown that when a latently infected carrier cow calves there may be transient excretion of *S. dublin* in the faeces, or from the vagina, and that congenital infection of the calf may occur. Similar cases were also identified by Hinton (1972*b*)

The majority of cows suffering from an *S. dublin* abortion will show evidence of active infection (Le Guilloux, 1968–75; Hinton, 1975). However, a small proportion do not develop agglutinins (Hinton, 1973*a*) and it is probable that these cases represent abortion in a latently infected animal which has no residual agglutinating antibodies in the serum. Support for the suggestion comes from the fact that most of these animals are only identified after the use of enrichment techniques (Table 2).

Transient vaginal excretion may occur in a faecal excretor at parturition (Hinton, 1974) and presumably this could occur if such a cow was to abort. Indeed, this could be the explanation for some of the cases identified by Hinton (1973*a*) in which there were diagnostic agglutinins in both serum samples but which did not appear to show any significant change either up or down.

Identification of recovered cases

The retrospective identification of cases may prove difficult as excretion of *S. dublin* in the faeces, milk and vaginal mucus is transient and usually ceases by the fourth week. Similarly the serum agglutinins frequently fall to low titres fairly soon after the abortion (Hinton, 1974).

Public health considerations

It is possible that an outbreak of salmonellosis in cattle is only suspected following the diagnosis of disease in man and in the context of salmonella abortion there are two particular situations in which this may occur. The first is that salmonella may be excreted in the milk for several weeks after abortion (Hinton, 1973*b*) and this could lead to an outbreak of milk-borne salmonellosis, while secondly there are reports of a salmonella folliculitis developing on the arms of veterinarians assisting an infected cow at calving (Williams, 1969; Pantekoek, Rhodes & Saunders, 1974).

CONCLUSIONS

S. dublin is not only a typical enteric pathogen in cattle but may also behave as a bacterial abortifacient. The organism may either cause abortion, or be associated with stillbirths or with the birth of normal healthy full-term calves. The clinical syndrome observed does not appear related to any specific property of the strain of *S. dublin* involved.

Selective salmonella media should be used for diagnostic purposes. SFB, incubated at 37 °C or 43 °C, is satisfactory for enrichment while DCA and BGM are suitable plating media.

The isolation of *S. dublin* from the products of conception, especially if it is in pure culture, coupled with significant changes in the agglutinating or complement fixing antibodies in the serum is diagnostic for a *S. dublin* abortion.

The isolation of *S. dublin* itself is not diagnostic as this may be possible when an active or a latent carrier cow aborts. The accurate identification of these cases depends on the examination of paired serum samples obtained within one month of abortion.

The retrospective identification of a case of *S. dublin* abortion by bacteriological or serological examination may prove unrewarding as excretion of the organism is often transient and the agglutinating titres usually decline to low levels after the fourth week.

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