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Sources of *Streptococcus dysgalactiae* in English and Welsh sheep flocks affected by infectious arthritis (joint ill)

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Abstract

In order to investigate sheep sources of *S. dysgalactiae* in flocks affected with joint ill, ten sheep flocks in England and Wales with laboratory-confirmed cases of infectious arthritis (joint ill) caused by *Streptococcus dysgalactiae* were visited during a disease outbreak while a further four flocks were visited during the lambing period in the year following an outbreak. A total of 5239 samples were collected for bacterial culture from 797 ewes and their 1314 lambs. *Streptococcus dysgalactiae* was isolated from nine of 894 samples (1%) on farms visited during an outbreak, and from 7 of 4462 samples (0.2%) collected in the year following an outbreak. The 16 samples from which *S. dysgalactiae* was isolated came from the vagina of 8 ewes, milk of 1 ewe, navel of 4 lambs, mouth of 2 lambs and nose of 1 lamb. In vitro testing of the survival of *S. dysgalactiae* on straw, hay and in water at different temperatures was performed and it was isolated from these substrates for up to 42, 35 and 0 days respectively.

Introduction

Streptococcus dysgalactiae subsp. *dysgalactiae* is a Lancefield Group C, alpha-haemolytic

streptococcus isolated only from animal species. In piglets it can cause arthritis, meningitis and endocarditis (Woods and Ross 1977) and has been identified in nasal, throat, tonsil and vaginal samples from healthy pigs (Jones 1976). In cows it has been identified as a cause of mastitis as well as being isolated from tonsil, oral and vaginal samples (Cruz-Colque and others 1993, Calvinho and others 1998). The organism has also been isolated from the cattle fly *Hydrotaea irritans* which is associated with the transmission of summer mastitis (Bramley and others 1985). Sporadically, *S. dysgalactiae* has been isolated as the primary disease causing agent in other animals such as neonatal mortality of puppies (Vela and others 2006). In sheep, *S. dysgalactiae* is the most common cause of outbreaks of polyarthritis, commonly known as joint ill, in lambs in England and Wales (Watkins and Sharp 1998). Affected lambs are usually less than 4 weeks old and the majority begin to show clinical signs at 10-14 days of age (Angus 1991, Watkins and Sharp 1998). Joint ill has been reported in the UK since the 1890's but the literature regarding the biology of *S. dysgalactiae* in sheep is limited. The organism has been isolated from the mouth and vagina from a small number of ewes (Cornell and Glover 1925, Anon 2006) and a milk sample from one ewe (Lacasta and others 2008). Environmental sources of the bacterium have not been investigated. The transmission routes for *S. dysgalactiae* are unknown although it is thought most likely to be via the umbilicus shortly after birth (Blakemore 1939, Angus 1991).

Many lambs in England and Wales are born indoors on straw bedding and remain indoors for at least 24 hours after birth so if bedding material became contaminated with *S. dysgalactiae* they could potentially be exposed for some time. There has been no previous research determining the ability of bedding materials to sustain *S. dysgalactiae* but research investigating the survival of primary mastitis pathogens such as *Streptococcus uberis* and *Escherichia coli* on both sterile and used bedding material (straw, wood chips and shavings) has shown they can sustain bacterial growth (Bramley 1982, Zehner and others 1986). *Streptococcus uberis* has been shown to survive in vitro on straw for at least 4 weeks (Mackey and Hinton 1990).

The aim of this study was to investigate potential sheep sources of *S. dysgalactiae* in flocks affected by joint ill, with the hypothesis that colonised ewes are a source of infection. An additional aim was to determine the in vitro environmental survival of the organism on materials commonly found in the indoor lambing environment.

Materials and methods

Animals

During the lambing seasons of 2008, 2009 and 2010 visits were made to 14 sheep flocks, ranging in size from 100-1800 ewes (mean 760 ewes) in England and Wales that had laboratory-confirmed cases of *S. dysgalactiae* joint ill in lambs. The case definition for an affected flock was that at least 2% of lambs demonstrated clinical signs consistent with joint ill (lameness, joint swelling, joint hot on palpation), and that *S. dysgalactiae* was isolated from joint fluid from at least one lamb. Ten of the farms had a joint ill outbreak in the year of sampling with disease prevalence ranging from 2-20%. On those farms, sampling was done within 1-8 weeks of the start of the outbreak, lambs ranged in age from 1-14 weeks, and both affected and unaffected lambs and their dams were sampled. The remaining four farms had joint ill outbreaks in the year prior to sampling with 5-20% prevalence; however in the year of sampling less than 0.1% of lambs were affected on any of these farms. On these farms lambs and their dams were sampled 0-48 hours after parturition.

In all flocks except one, lambing occurred indoors. Ewes in indoor-lambing flocks were kept in straw-bedded group pens until shortly after parturition when they were transferred with their lamb(s) to a straw-bedded individual pen where they remained for 24-48 hours. For the majority of flocks these pens were not cleaned or disinfected between occupations. Ewes and lambs were then transferred to indoor straw-bedded group 'nursery' pens for a further 1-5 days before turn-out into fields. The remaining flock kept the pregnant ewes outdoors in a dirt yard adjacent to the barn. Shortly after parturition each ewe and her lamb(s) were transferred to indoor straw-bedded individual pens and then managed in a similar manner to the indoor-lambing flocks. In all flocks ewes were fed ad-lib hay and/or silage and a rationed quantity of concentrate feed.

Sample collection

Samples for bacteriological culture were taken from a variety of sites from lambs and ewes. From the first four farms that were visited, all of which were visited during a joint ill outbreak, samples were collected from the mouth, nose and skin around the navel as well as the ear tag, tail dock and castration wound or site of lambs (n = 39 lambs), and the vagina, mouth, nose, wool, skin and teat of ewes (n = 16 ewes). Subsequently from the remaining 10

farms samples were collected only from the mouth and skin around the navel of lambs and the vagina, nose and milk of ewes. Samples were collected using swabs with Amies charcoal transport medium (Sterilin Ltd) and most were cultured within 24 hours of collection. The milk samples were collected into sterile 7 ml disposable plastic screw top containers (Bijou, Sterilin Ltd) and frozen at -20°C until cultured (Schukken and others 1989).

Bacteriology

Swabs collected from the first 4 farms that were visited (n = 346 samples) were streaked onto Columbia agar plates (Oxoid) supplemented with 5% sheep blood (TCS Biosciences), 5mg/L oxolinic acid and 10mg/L colistin sulphate (Streptococcus selective supplement, Oxoid). Samples collected from subsequent 10 farms (n = 4983 samples) were streaked onto the same plates as above which had been further supplemented with 1g/L aesculin (Sigma) and 0.5g/L ferric citrate (Sigma). All plates were incubated at 37°C for 24 hours. *S. dysgalactiae* was identified as small grey alpha-haemolytic, aesculin-negative colonies. Suspect colonies were sub-cultured until a pure culture was obtained and then subjected to catalase testing, Lancefield Grouping using a commercial grouping kit (Pro-Lex; Pro-Lab Diagnostics) and API testing (API Strep; Bio-Merieux Inc) to confirm the presence of *S. dysgalactiae* in the sample.

Survival on straw, hay and in water

Three materials found in housed lambing environments were chosen as growth substrates for *S. dysgalactiae*: straw, hay and water. The growth of the bacteria on the materials at 3 different temperatures (37°C, room temperature and 4° C) was determined.

To prepare the inocula, *S. dysgalactiae* was cultured for 24 hours on blood agar and then one isolated colony was inoculated into sterile Brain Heart Infusion broth and incubated for 12 hours at 37°C. After incubation the broth culture was diluted at 1:100 in 0.85% sterile saline (SS). To determine the initial number of colony forming units per millilitre in the initial inoculum, duplicate plate counts were performed using 10-fold serial dilutions cultured on blood agar.

To prepare the materials for inoculation, straw and hay samples were chopped into 1cm pieces and sterilised by autoclaving. Approximately 1g of material was aseptically transferred to a sterile Whirl-pak™ (Nasco) bag and 4ml of SS added; a total of 20 bags of each material

were prepared. Tap water was sterilised by autoclaving and 4 ml was aseptically transferred to a sterile 5 ml bijoux; a total of 20 bijoux were prepared.

After equilibration at room temperature, one ml of diluted broth culture was aseptically transferred to each sample bag/bijou which were then sealed and incubated at 37°C, room temperature or 4°C. Sampling was done on 8 occasions at 24 hours, 48 hours, 7, 14, 21, 28, 35 and 42 days after inoculation. At each time point two bags each of straw and hay and 2 bijoux of water were identified. Five ml of SS was added to each bag of straw and hay. The samples were resealed and shaken vigorously to mix the contents. One ml of solution was then withdrawn per bag or bijou and used to perform 10-fold serial dilutions in triplicate onto blood agar. Blood agar plates were incubated for 24 hours at 37°C and bacterial colonies were counted at the dilution that individual colonies could be easily distinguished from each other. The counts for the 3 plates at that dilution were averaged to obtain the number of colony forming units per ml (CFU/ml). Once each bag/bijou was sampled it was discarded.

In addition 2 control samples were used per material per temperature to ensure no contamination occurred; 1 ml of SS was added to the control bags/ bijoux but no inoculum added and these were sampled at 24 hours and 35 days as above.

This research was approved by the Royal Veterinary College Animal Ethics Committee.

Results

Isolation of *S. dysgalactiae* from lambs and ewes

A total of 14 flocks were visited from which 1314 lambs and 797 ewes were sampled. A total of 5239 samples were collected of which 16 yielded *S. dysgalactiae*.

Amongst the 10 flocks which were sampled during an outbreak of joint ill, 91 lambs and 125 ewes were sampled and a total of 894 samples were collected (Table 1). *S. dysgalactiae* was isolated from nine of the samples (1%). Amongst the four flocks which were sampled in the year following a joint ill outbreak, 1223 lambs and 672 ewes were sampled from which 4462 samples were collected and *S. dysgalactiae* was isolated from seven (0.2%; Table 1). No individual animal had *S. dysgalactiae* isolated from more than one site.

In-vitro survival of *S. dysgalactiae* on straw, hay and in water

The medium that supported *S. dysgalactiae* for the longest period of time was straw – the organism was isolated from straw for up to 35, 21 and 42 days when incubated at 37°C, room temperature and 4°C, respectively (Figure 1). Survival times on hay were shorter with the longest survival of up to 35 days when incubated at 4°C (Figure 2). *S. dysgalactiae* was not isolated from water at any time point after inoculation.

Discussion

In this study, *S. dysgalactiae* was isolated from the vaginal tract of a small number of ewes, which has also been reported previously (Cornell and Glover 1925, Anon 2006). Similarly it has been isolated from the vagina of sows and cows (Jones 1976; Cruz-Colque and others 1993; Calvinho and others 1998). This suggests the organism may live as a commensal in the vaginal tract and if so, the lambs of colonised ewes may become exposed during birth. If it is subsequently shed in vaginal discharges then the resulting environmental contamination might allow other lambs born in the area at a similar time to become exposed. This study demonstrated that *S. dysgalactiae* could survive in-vitro on straw for up to 42 days, which is approximately the length of the lambing period on many farms. However in this study the bedding on farms was not tested for the presence of *S. dysgalactiae* and it is unknown how the presence of urine, faeces and birth fluids on contaminated bedding, as would be expected in the lambing environment, would affect its survival. Isolation from the superficial skin of the navel of a small number of lambs in this study suggests the bacteria were present in the lambs' environment or that they can survive on this site for some time.

Despite collection of a large number of samples for bacteriology *S. dysgalactiae* was isolated from only 1% of samples collected at the time of a joint ill outbreak and 0.2% of samples in flocks that had an outbreak in the previous year. Similarly Lacasta and others (2008) did not isolate *S. dysgalactiae* from the nose, ear or vagina of 30 ewes who had lambs with *S. dysgalactiae* joint ill. The low frequency of isolation suggests carriage of the organism is relatively infrequent. Nevertheless it is possible that a small number of colonised ewes could expose a large number of lambs to the organism depending on the stocking density, the timing and frequency of lambings, and the timing, frequency and depth of re-bedding.

In humans, early-onset Group B streptococcal (GBS) infections are transmitted from mother to child during parturition so screening for GBS is achieved by antenatal vaginal culture of

the mother (Verani and Shrag 2010) and in this study it would have been ideal to have sampled the ewes immediately before or after parturition in the year of a joint ill outbreak. However, due to delays in the diagnosis of joint ill on farms and notification of the investigators there was a time-lag from the start of a joint ill outbreak until sampling was done so the ewes were sampled 1-14 weeks after parturition. It is possible this delay might have reduced the isolation rate of *S. dysgalactiae*. Further research involving consecutive sampling of the vagina of colonised ewes would be necessary to evaluate the persistence of vaginal carriage and the changes in carriage around parturition. An attempt was made to sample ewes and lambs within 48 hours of parturition by targeting flocks that had a large joint ill outbreak in the previous year but unfortunately in the year of sampling the prevalence of joint ill in these flocks was very low which may be why the isolation rate was so low from these flocks.

The organism was identified in the milk of one ewe and similarly Lacasta and others (2008) tested milk samples from 95 ewes and isolated *S. dysgalactiae* from the milk of 1 ewe whose lamb subsequently developed joint ill. This may be a source of infection for some lambs. Tonsillar carriage of *S. dysgalactiae* has been reported in pigs (Jones 1976) and cattle (Cruz Colque and others 1993) and it is possible that these may be an important site of carriage in ewes as well. Tonsils were not sampled in this study and neither were faeces; however the growth of *S. dysgalactiae* is inhibited by the presence of bile salts suggesting it is unlikely to survive in the intestines or faeces. Devriese et al (1994) failed to isolate *S. dysgalactiae* from the intestines or faeces of swine even though it was present in their tonsils.

It is possible that the main source of the bacterium is from somewhere other than colonised ewes but in the indoor lambing environment there are likely to be very few sources of bacteria apart from the sheep, the bedding, feed and water. In this study the organism survived in vitro on straw and hay for a maximum of 42 and 35 days respectively and not at all in water. Given that straw and hay are made during the summer and then stored for at least 6 months before lambing, it is highly unlikely that these could be a primary source of *S. dysgalactiae* infection during lambing. Furthermore anecdotal reports from Veterinary Investigation Centres, where lambs born to ewes from one group had joint ill whereas lambs born to separate groups of ewes on the same farm did not, lends support to the theory that colonised ewes are the main source of *S. dysgalactiae* on farms (Anon 2006, 2007).

In this study there was variation in the sampling strategy between the first four farms visited and the subsequent 10. Initially samples were taken from six sites on lambs and seven on ewes but in the interests of practicality this was rationalised to two and three sites respectively. It is possible more isolations of *S. dysgalactiae* would have been made if more sites had been sampled. Ideally the tonsils of ewes should have been sampled but this is very difficult to do in the live animal.

Assuming the main source of *S. dysgalactiae* in affected flocks is colonised ewes, and given that *S. dysgalactiae* joint ill is likely to be a contagious disease, simple hygiene procedures aimed at limiting the possibility of transmission from ewe to lamb and ewe to ewe may be beneficial. For example wearing clean gloves during assisted lambing, promptly removing placentas and birth fluid-contaminated straw after lambing, re-bedding relatively frequently and cleaning or re-bedding individual lambing pens between occupations would seem sensible. If some groups of ewes are known to produce lambs with joint ill, lambing these ewes separately from other groups of ewes could be practiced. In affected flocks where batch-lambing takes place on non-overlapping dates, it would seem sensible to recommend that batches of ewes lamb in separate areas (outdoor-lambing flocks) or the lambing shed is emptied and disinfected between batches of lambing ewes (indoor-lambing flocks). As *S. dysgalactiae* was isolated from the mouth of two lambs, in affected flocks it would seem prudent to recommend disinfecting stomach tubes between lambs. However the effectiveness of these recommendations at decreasing the incidence of *S. dysgalactiae* joint ill is unknown.

Of the 9 ewes from which *S. dysgalactiae* was isolated from the vagina or milk, only one had a lamb with confirmed joint ill suggesting not all lambs exposed to the organism will subsequently develop joint ill; whether they develop it is likely to be related to the level and timing of exposure, level of immunity, efficacy of any prophylactic treatments and possibly environmental factors. Characteristics of the lambs themselves in terms of birth-weight, gender and litter size did not appear to have any major influence on whether they develop joint ill (Rutherford 2012). In lambs the umbilicus is proposed to be the most likely route of entry of *S. dysgalactiae* (Blakemore 1939; Angus 1991) although the digestive pathway has also been suggested (Lacasta and others, 2008).

In summary, it appears that carriage of *S. dysgalactiae* in sheep is uncommon but it is likely

that colonised ewes are an important source of *S. dysgalactiae* infection and that excretion in infected vaginal fluids or potentially other secretions may result in environmental contamination and transmission to lambs. In a small number of cases ingestion of infected milk may also be a source of infection. The organism may survive on straw or hay for up to 5-6 weeks. There is still much that is unknown about this disease and further research is necessary to increase our understanding of it.

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Table 1. Isolation of *S. dysgalactiae* from various sites in or on lambs and ewes from 10 flocks affected with *S. dysgalactiae* joint ill in the year of sampling and 4 flocks affected in the previous year.

Time of sampling in relation to joint ill outbreak	Age of sheep	Sample site	Number of samples	Number of samples positive for <i>S.dysgalactiae</i>
Within 1-8 weeks of the start of the outbreak (10 flocks)	Lambs (n=91)	Mouth	91	1 ^a
		Navel	91	2 ^a
		Nose	39	1 ^a
		Ear-tag area	39	0
		Tail dock area	39	0
		Castration area	39	0
	Ewes (n=125)	Vagina	125	4 ^b
		Milk	125	1 ^c
		Nose	125	0
		Wool	16	0
		Skin	16	0
		Teat	16	0
		Mouth	16	0
Total			894	9
Joint ill in previous year (4 flocks)	Lambs (n=1223)	Mouth	1223	1 ^d
		Navel	1223	2 ^d
	Ewes (n=672)	Vagina	672	4 ^c
		Nose	672	0
		Milk	672	0
Total			4462	7

Grand Total	5329	16
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^a Lambs also had joint ill

^b One ewe had twin lambs, one of which had joint ill and the other did not. A second ewe had a lamb which died for unknown reasons. The remaining 2 ewes had lambs which did not have joint ill

^c Lambs born to these ewes did not have, or develop, joint ill

^d Lambs did not have, or develop, joint ill

Figure 1. In-vitro survival of *S. dysgalactiae* on straw incubated at either 4°C, room temperature or 37°C.

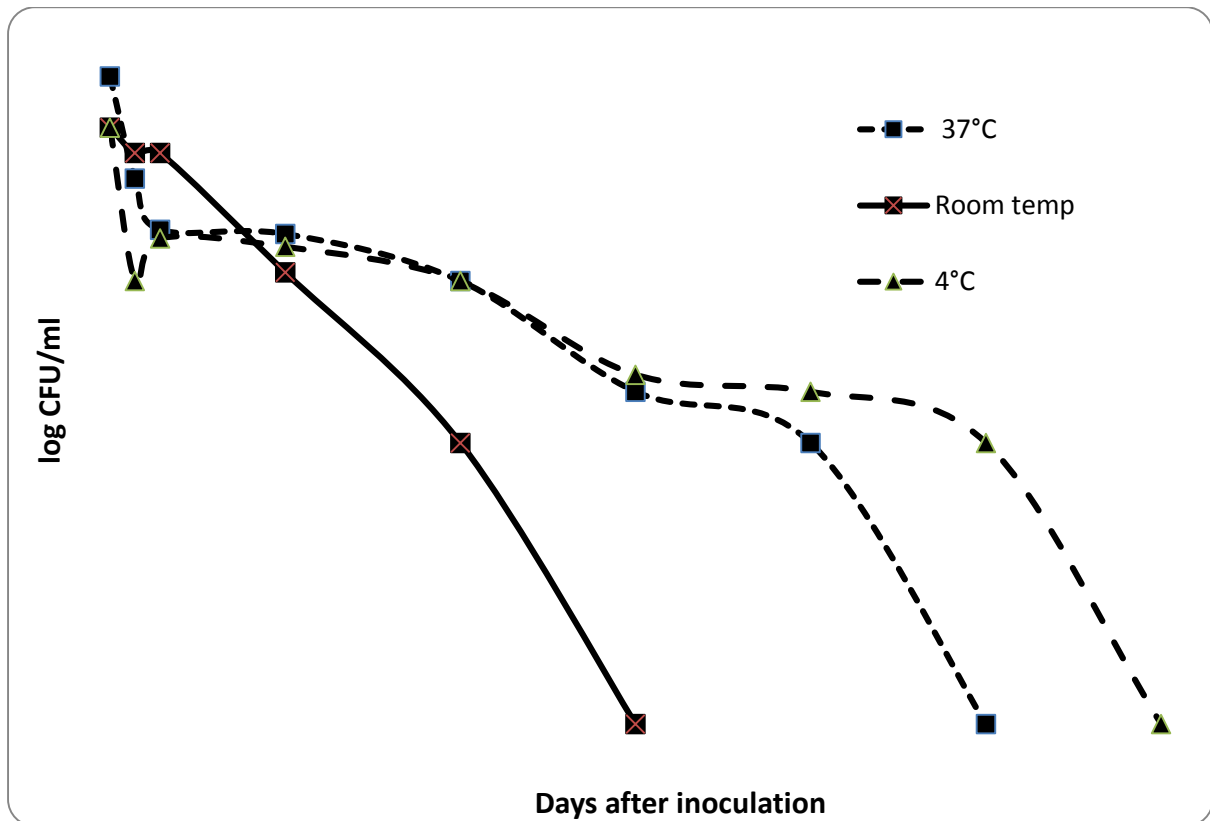


Figure 2. In-vitro survival of *S. dysgalactiae* on hay incubated at either 4°C, room temperature or 37°C.

