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Transmission of Mycoplasma Dispar among a Succession of Newborn Calves on a Dairy Farm

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Tanskanen, R.: Transmission of Mycoplasma dispar among a succession of newborn calves on a dairy farm. Acta vet. scand. 1987, 28, 349–360. – Transmission of Mycoplasma dispar among 33 newborn calves (nos. 1–33) on a dairy farm, transferred in order of birth into individual pens in a separate calf rearing room during a period of 50 days, was investigated. The first calf (no. 1) was deliberately infected. Weekly nasal swabs were taken 9 times.

Twenty-eight susceptibles (data for 4 calves are excluded because of omissions in sampling) were found to be positive for M. dispar on average 8.6 days (the range 5 to 15 days) after the start of exposure. Among the first 7 (nos. 2–8) and the latter 21 susceptibles (nos. 9–10 and 15–33), the infection was first detected on average after 12.1 and 7.4 days, respectively. The interval measured (from the start of exposure to the first detection of infection) comprised 3 parts: the transmission time proper, the latent period and the delay in detection of positivity due to the sampling interval. The variation affecting the first 2 parts of the measure, and especially the transmission time, clearly must account for the above difference between the 2 study phases; an association with the density of infective calves was apparent. The last component of the measure was distributed randomly and its lenghtening effect on the figures was estimated at 2 to 3 days. By subtraction, the average period preceding detectable colonisation in the latter part of the study was approximated as 4 to 5 days. Through another approach, estimation by Reed-Muench calculation, the figure was 4.7 days. The existing knowledge of the usual lengths of latency – with the present limited variation among the intervals measured – indicates that the lag in transmission, i. e. the transmission time proper, in the latter phase of the study was unlikely to exceed a few hours; the major part of the average 4 to 5 days interval was thus accounted for by latency. The principal mode of transmission was inferred to have been air-borne, sometimes over several meters.

Forty-five adult cows, sampled after the delivery, including most dams of the calves studied, were all negative for M. dispar; M. bovirhinis and Acholeplasma laidlawii were isolated from 8 and 3 cows, respectively.

Mycoplasma infections; mycoplasmosis in cattle; respiratory diseases of cattle; epidemiology; disease transmission.

Introduction

Transmission of Mycoplasma dispar has been investigated both in experimental (i. e. rather carefully controlled) conditions and in the natural setting of both calf transportation and calf rearing (*Tanskanen* 1987b, c). The results have indicated a relatively high transmissibility for the agent

in conditions allowing physical contact between the source and susceptibles. A rather short period of potential contact sufficed to establish infection in part of the susceptibles both in the experimental setting and during calf transportation. On the other hand, in experimental conditions the spread of infection was shown to be delayed when the distance between the source and susceptible calves was extended to about 1.3 m.

In the present study the spread of M. dispar infection was investigated among a series of newborn calves on a dairy farm. The calves were transferred shortly after their birth to a separate room, into which M. dispar infection had been introduced with the first calf, and the process of catching the infection by the newcomers was monitored. In connection with the deliveries of these test calves also the majority of the dams were investigated for presence of respiratory mycoplasmas. Nasal swabbing was used as the method of detecting and measuring the degree of colonisation.

Material and methods

Susceptible calves

A total of 32 newborn Ayrshire calves, 26 males and 6 females, (nos. 2–32 in order of birth), born during a period of 50 days in December 1983 and January 1984 on a closed dairy farm belonging to the Agricultural Research Centre in Jokioinen, constituted the group of susceptible calves. The assumption of susceptibility is based on both the unlikelihood, and a current lack of all evidence indicating, that the parent cows in the infected herds would act as source of infection for their calves. Twenty-eight of the calves were transferred on the day of delivery from their dams directly to a separate room in the near-by calfhouse to be subjected to the transmission study; 4 calves (nos. 14, 20, 30 and 33) stayed overnight in individual pens close to their dams before the transfer. The susceptible calves had no contact with older calves on the farm before being subjected to the transmission study. The calves received 6 feeds of colostrum (each 1.5 l) during their first 2 days.

Adult cattle

Altogether 45 adult Ayrshire cows were sampled shortly after calving. These consisted of 29 cows delivering 30 of the study calves, and of 16 cows which had calved earlier, in November 1983. The average age (SD) of the cows sampled was 4.4 (2.0) years within a range of 2.0–10.1 years.

Accomodation of calves

The experiment took place in a separate room (326 m^3 in size) located in the calfhouse. Calves were placed in individual pens (1.0 m × 1.2 m in size) with 3 solid sides, 0.9 m in height, and an open front side with equipment for feeding. The layout of the pens is shown in Fig 1. Passages, 1.6 m wide, separated each 2 rows of pens facing each other. Straw was used for bedding for the first 2 weeks of rearing, thereafter no bedding was used. The room was centrally heated. During the first week of rearing the calves the pens were equipped with heat lamps for additional heating.

Good air hygiene was considered to prevail in the room. Air-conditioning was controlled by an electrical ventilation system (Fristamat Disco* with one supply unit type PDA-608). The maximal air intake supplied by the system was 7000 m^3/h. The thermostat of the ventilation system was adjusted to temperatures between 17°C and 20°C. The temperature and relative humidity (RH) values were not recorded during the study but

* Suomen Karjatilatarvike Oy / Nordisk Ventilator Co. A/S.

were available from the corresponding period 1 year later, in December 1984 and January 1985. Then the mean daily maximum and minimum temperatures and variations (SD) were 18.5 (0.8) and 16.9 (1.4) °C, respectively. RH varied from 35 to 75 % with a mean of 60.9 %. No changes in air-conditioning had taken place in-between. Also the number of calves accommodated in the room was approximately the same as during the study period in 1983–1984.

The room was cleaned and disinfected and then left empty for a period of 3 weeks before the entry of the first study calves. The calves were taken care of by a single animal handler who wore separate protective clothing when working in this room. The handler started from the youngest and latest arrivals through to the oldest and earliest arrivals in strict order. Three feeds a day were given. Individual buckets were used for feeding. The calves were subjected in parallel to a feeding experiment concerning the effect of addition of group B vitamins to the normal diet of newborn calves.

Arrangements of the study

The calf (no. 1) preceding in birth order the first susceptible calf (no. 2) was allowed, after its birth, to stay overnight (12 h) in short-distance contact (in a small pen adjacent to a larger pen) with a group of older calves of the farm known to be infected with M. dispar. Calf no. 1, expected to have been infected by this exposure, was transferred to the study room (on day 0) to act as the primary source of infection. The susceptible calves arrived in the study room in order of birth. The schedule of the arrival – with its irregularities – of individual calves in the study room is given in Table 1. The last calf (no. 33) in the succession to be studied was placed in the room 50 days after the arrival of calf no. 1. Position of the calves in the room, i. e. their placing in the pens, is shown in Fig. 1.

Sampling

The first sampling took place on day 7. Calf no. 1 and the first 2 of the susceptible calves (nos. 2 and 3) had already been brought into

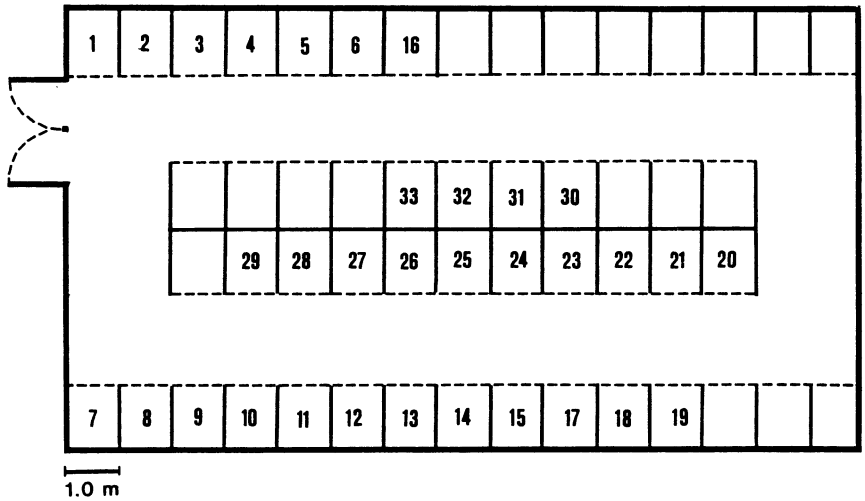

Figure 1. The layout of pens with the position of the 33 newborn calves (nos. 1–33) subjected to the follow-up survey in the study room.

the study room 7, 6, and 5 days earlier, respectively (Table 1). The following samplings (II–IX) took place at approximately weekly intervals during the next 8 weeks. As can be seen from Table 1, samplings II to IX involved 6, 8, 10, 23, 25, 31, 33, and 31 calves, respectively (calf no. 1 is included in these figures). Before the last sampling, calves nos. 1 to 3 were removed from the room. For samplings I to IX age ranges (with the averages) for the calves susceptible at arrival (calf. no. 1 omitted) were 5–6 (5.5), 1–8 (5.2), 5–20 (10.9), 7–27 (15.4), 1–34 (14.0), 2–41 (17.6), 1–48 (20.5), 6–55 (26.2), and 13–51 (31.3), respectively.

Twenty-eight of the 46 adult cows were sampled on the day of delivery, 15 were sampled on the day after delivery, 1 and 2 of the cows on days 2 and 3 after delivery, respectively.

The nasal swab specimens were taken in a manner previously described (*Tanskanen* 1984). For the samplings IV and V the order of obtaining the specimens was from the youngest calf to the oldest. At other times instructions, apparently inadequately emphasized, were not strictly followed and partial sampling in the reverse order took place. An effort was made, however, to avoid indirect transfer of the agent in the connection with sampling. The specimens (tubes containing mycoplasma broth with swab) were either transported on the day of sampling the 120 km distance to the laboratory or were preserved frozen at –20°C on the farm, for 2 weeks at the most, before transport. Culturing of the specimens occurred immediately on arrival in the laboratory or after a storage of a few months at –70°C.

Clinical observations

Rectal temperatures were recorded weekly. Observations of couching or other symptoms were made and recorded in connection with the daily handling of calves.

Culture and identification of mycoplasmas

The medium used – a modification of glucose calf-serum medium described by *Gourlay & Leach* (1970) – as well as the methods of culturing and identification of mycoplasmas have been described earlier (*Tanskanen* 1984).

'Prepositive period'

The technical concept 'prepositive period' used in this paper refers in the majority of cases to the period from the date of arrival of the calf in the room to the date of first detection of M. dispar in the nasal specimen; in the case of calves nos. 2 to 6 the prepositive period refers to the period from the date of first detection of calf no. 1 (first source of infection in the room) to be positive to that of first detection of M. dispar in those calves.

Mathematical approach

The Mann-Whitney U test was used for statistical analyses of differences in prepositive periods. A modified application of the Reed-Muench method (*Reed & Muench* 1938) was further employed to exclude the delay in sampling from the time measure: grading was made by temporal instead of titer-related differences to estimate the interval between the start of exposure to the 50 % detection of established colonisation.

Results

Table 1 shows the appearance and development of titers for M. dispar among the 33 calves transferred to the study room in order of their birth during a period of 50 days and sampled at approximately weekly intervals beginning from the start of the follow-up.

All the susceptibles were found to be infected within 5 to 26 days after their arrival; if the beginning of the exposure among the first 5 susceptibles is conservatively assigned to the date of finding calf no. 1 to be infected, then the longest prepositive period (in calves nos. 3–5) will be 15 days. Taking into account the latter qualification the average length of the prepositive period (with SD) for 28 susceptibles will be 8.6 days (3.0) (the results for calves nos. 11–14 are omitted due to an excess delay in their first sampling; see Table 1).

A shortening of the prepositive period with an increase in the number of calves – and accordingly in the number of infected calves – took place rapidly and seemed to remain so throughout the main of the follow-up. This occurred approximately after 1/4 of the calves had been introduced to the study. This development is presented in a graphical manner by the overall result given in Table 2. For calves nos. 2–8 the average prepositive period (with SD) was 12.1 (3.1) days, whereas for calves nos. 9–10 and 15–33 it was 7.4 (1.9) days. The difference was significant ($p < 0.01$). There was a significant negative correlation ($r = -0.57$, $p < 0.01$) between the phase of the calf entering the study (expressed as days passed since the original source calf for the infection, calf no. 1, was detected to be positive) and the length of the prepositive period.

All the 11 susceptibles which were initially sampled at 1 to 3 days after the transfer into the study room and 1 calf sampled 4 days after transfer were negative for M. dispar. Two of 5, 5 of 7 and 6 of 6 calves, sampled for the first or second time on days 5, 6, and 7 after arrival, respectively, had already turned positive. Among 3/4 of the calves, i. e. the latter part starting with calf no. 9, the corresponding figures for the proportions of positives on days 5, 6, and 7 were: 2/2, 5/5, and 6/6, respectively. None of the calves in this group were found negative on samplings after day 5. When calculation using the Reed-Muench method was applied to the data on prepositive (and confirmed negative) periods for the latter 3/4 of calves (nos. 9, 10 and 15–33), the dividing line of 50 % detectable positivity (and apparent negativity) among the intervals examined was set at 4.7 days. The comparison with the figure 7.4 days, i. e. the mean of prepositive periods for these calves, suggests that the delay in the detection of established infection caused by the sampling interval was in the latter part of the study on average 2 to 3 days. The delay of 2 to 3 days appears reasonable also in view of the actual sampling by weekly intervals (with the first sampling being distributed randomly during the first 7 days) and also by the fact of the frequent high titers of the first positive specimens.

The pattern of placing newcomers into the pens (see Fig. 1) was principally that of filling consecutive pens in a row and then switching to another row. The placement of calf no. 16 was an exception. This arrangement allowed observations both of transmission within a particular row or from one row to the other. In the first phase of the follow-up study the order of becoming recognizably infected was not the most likely one, in that calf no. 6 preceeded calves nos. 3, 4 and 5, which were located in the pens lying between calf.no. 1 – the original source of infection – and calf. no. 6 (see Fig. 1). Within the row, on several occasions pairs or more of calves in adjacent pens (3, 4, 5; 7, 8; 9, 10; 17–19; 20–23; 24, 25; 26–28; 30, 31; 32, 33) appeared to have caught the infection almost simultaneously, which strongly suggests a type of transmission other than through a chain of physical calf-to-calf contact. Transfer of the infection from one row of pens to another also appeared to have

Table 1. Titers for M. dispar in nasal swab specimens obtained at weekly intervals from young calves, 32 at most, that were transferred in order of birth to the study room and subjected to natural infection.

Calf no.*	Arrival in study room prior to first specimen (days)	Titer (log_{10} ccu) on samplings I to IX/days post start of experiment**								
		I/7	II/13	III/21	IV/28	V/35; 38	VI/42	VII/49	VIII/56	IX/63
1***	7	0	4	5	8	7	6	6	4	–
2	6	0	0	5	6	7	7	5	0	–
3	5	0	0	0	5	7	7	7	5	–
4	1	–	0	0	7	7	5	5	6	5
5	1	–	0	0	7	6	8	6	5	5
6	1	–	0	3	7	6	7	6	5	6
7	5	–	–	0	3	7	6	6	5	5
8	5	–	–	0	6	6	6	7	6	5
9	7	–	–	–	5	5	5	5	5	6
10	7	–	–	–	3	6	6	7	5	3
11	15	–	–	–	nd	5	6	6	6	5
12	14	–	–	–	nd	6	5	7	7	6
13	13	–	–	–	nd	6	6	6	4	7
14	12	–	–	–	nd	6	7	6	6	6
15	9	–	–	–	–	6	6	6	6	6
16	6	–	–	–	–	0	5	6	5	6
17	6	–	–	–	–	5	5	7	6	6
18	6	–	–	–	–	3	6	7	7	5
19	5	–	–	–	–	3	6	7	7	4
20	4	–	–	–	–	0	5	6	7	3
21	3	–	–	–	–	0	6	6	5	7
22	2	–	–	–	–	0	5	6	7	5
23	1	–	–	–	–	0	6	6	5	5
24	2	–	–	–	–	–	0	5	6	6
25	2	–	–	–	–	–	0	7	7	6
26	7	–	–	–	–	–	–	2	5	0
27	7	–	–	–	–	–	–	3	6	5
28	6	–	–	–	–	–	–	6	3	4
29	3	–	–	–	–	–	–	0	5	7
30	1	–	–	–	–	–	–	0	6	7
31	1	–	–	–	–	–	–	0	7	7
32	7	–	–	–	–	–	–	–	4	6
33	6	–	–	–	–	–	–	–	4	5
No. of calves sampled		3	6	8	14	23	25	31	33	30

* Calves nos. 1, 13, 19, 29 and 32 were 1 day old when subjected to experiments; other calves were transferred only a few hours after the birth.

** On sampling V calves nos. 1–7 and 16 were sampled on day 35; others were sampled on day 38.

*** Calf 1, deliberately exposed after birth to M. dispar by contact with infected older calves, acted as a source of infection to first susceptibles.

taken place without delay, showing no significant difference from the within-row-transmission. The first 2 calves in each new row were found to be infected post arrival as follows: calves nos. 7 and 8 on day 12; calves nos. 20 and 21 on days 8 and 7, respectively. These figures do not differ markedly from the average figures for the groups of calves presented in the preceding paragraph.

The rise of the titers to the levels of 5 to 7 $\log_{10}$ ccu took place rapidly after the first detection of infection and the high levels in the great majority of cases were maintained throughout the follow-up period; calves nos. 3 and 26 with their abrupt zeros in the last specimen were exceptions and raised the question of the adequancy of these specimens. As shown in table 2 the titers for M. dispar at the first detections varied from 2 to 7 $\log_{10}$ ccu, having a mean of 5.0. The mean titers (with SD) for 32, 26, 22, 17, and 7 susceptible calves at the next 5 successive samplings after each first detection of the infection were 6.0 (0.9), 6.1 (0.9), 6.0 (1.1), 5.5 (0.7), and 4.9 (0.9) $\log_{10}$ ccu, respectively.

No other mycoplasmas apart from M. dispar were isolated from the calves surveyed during the study period.

Sporadic cases of coughing were first recorded approximately 4 weeks after the start of the follow-up. Thereafter, intermittent mild couching was recorded more regularly, with increasing frequency towards the end of the follow-up period. The overall incidence of couching largely equalled the prevalence at the end of the study and was roughly estimated to be one half to 3/4 of the calves. Couching usually started 2 to 3 weeks after the calf had arrived in the study room. Temperatures remained normal in all calves. Slight diarrhea was observed in 10 calves.

All 46 adult cows were negative for M. dispar. M. bovirhinis and Acholeplasma laidlawii were isolated from 8 (17.4 %), and 3 (6.5 %) of the cows, respectively, in titers of 1 to 3 $\log_{10}$ ccu.

Discussion

The transmission time, defined as the interval from the start of exposure to the establishment of infection following a receipt of mycoplasmas, i. e. a lag in transmission, has been the measure of the basic interest in the present study. The event of transmission mediated by continuous or repeated exposures is naturally often extended in time beyond the first establishment of infection. Of special interest is the negative measure, i. e. the length of time when the calves remained uninfected. This can, however, only be estimated indirectly and relatively as part of a combined measure which also includes the latent (prepatent) period of infection. Such a combined measure was also used in a previous experimental study (*Tanskanen* 1987b). In the present study, the method of sampling the calves at weekly intervals introduced a third component: namely, the length of time by which the detection of the infection was postponed due to infrequent, although regular, sampling and which seems to complicate the estimation of any nonrandom determinant-dependent variation in transmission time. (Although this component was of some influence also in the earlier experimental study referred to, its effect in this present survey has been systematic and clearly larger.) The estimations of relative transmission time do not seem, however, to have been seriously compromised by the indirect method applied. The delay in testing is assumed to be a random variable. Also the length of the latent period, although naturally influenced by the intensity and duration of the infective exposure, i. e. by the cumulative dose, is, as a host determinant, taken to be randomly distributed. The combined, 3-component measure used

Table 2. The date and titer of first detection of M. dispar in the succession of newborn calves after their arrival in study room for exposure to natural infection.

Calf no.	Arrival after detection of calf no. 1 positive*	Titer for M. dispar (log_{10} ccu) Days post arrival in study room**										
		5	6	7	8	9	10	11	12	13	14	15
2	0				5							
3	0											5
4	0											7
5	0											7
6	0				3							
7	3								3			
8	3								6			
9	8			5								
10	8			3								
11***												
12***												
13***												
14***												
15	16					6						
16	16									5		
17	19		5									
18	19		3									
19	20	3										
20	21				5							
21	22			6								
22	23		5									
23	24	6										
24	27					5						
25	27					7						
26	29			2								
27	29			3								
28	30		6									
29	33						5					
30	35				6							
31	35				7							
32	36		4									
33	37			4								
No. of positive calves		2	5	6	5	3	1	0	2	1	0	3
Mean titer		4.5	4.6	3.8	5.2	5.3	5.0	–	4.5	5.0	–	6.3

* Calf no. 1 acted as the initial source calf (see Table 1).

** For calves nos. 2–6 the day when calf. no. 1 was first detected positive is assigned as the start of their exposure (see Table 1). Counted from the arrival in the study room M. dispar was first detected in calves nos. 2–6 on days 20, 26, 16, 16, and 9, respectively.

*** The results for calves nos. 11–14 are ommitted due to the excess delay in their sampling (see Table 1).

has been named, for purpose of the present study, the 'prepositive period'.

A separate question is how the interval from the start of the potential exposure to the establishment of detectable colonisation is devided into the transmission time proper and the latent period. The latent period – in accordance with the related incubation time – is, as already referred to, obviously influenced by such factors as the level, duration and the mode of the infective exposure (by infective dosis in a broader sense) and the susceptibility of the host. In short time exposures of 4 calves in the experimental study (*Tanskanen* 1987b) the recorded latent times for 3 of them were at least 3 to 4 days. In calf no. 1 of the present study, also exposed only for a limited time, a latent period of at least 7 days was observed. Also after experimental intranasal inoculations of M. dispar latent periods, with respect to nasal swabbing, of 1 to 5 days have been recorded; in these experiments the period of the latency seemed to be roughly inversely related to the number of organisms inoculated (*Tanskanen,* unpublished data). The comparison of the period of latency in calf no. 1 with the apparent lack of long latencies among the calves during the latter phase of the present study also seems to lend further support to the suggestion of this reversed correlation. By Reed-Muench calculation the estimated average negativity period for the latter 3/4 of calves in the present follow-up study was only 4.7 days. (The precision of the figure is low because of the low number of calves examined in classes detected positive on days 4 and 5. However, an application of the same method to the cumulated data from 3 and 6 days classes also suggests a 4 to 5 days negativity period). This result, and the implications of the above data, suggest that these conditions contributed to a shortening of the transmission time proper to near 0, which means that the calves became infected with high regularity within the first day or even the first hours after their transfer to the study room. The inference of a very short transmission time proper is additionally supported by the suggestion that the intensity and duration of an air-borne exposure in the latter phase of the study had reached a level at which even the latent periods, obviously correlated in length with the transmission time proper, likely had fallen into the virtually shortest dimensions attainable by this mode of natural exposure. The narrow range of variation of the prepositive periods recorded supports this notion.

It seems quite clear from the present results that transmission among the majority of the susceptibles did not occur through calf-to-calf contact. This result is of considerable interest, because in the experimental study physical contact was observed to be a prerequisite for the most readily occurring transmission (*Tanskanen* 1987b). How then is the difference to be explained? Within the present survey a variation in the prepositive period, and hence by inference in the transmission time, was observed. Transmission was in the beginning slower but at the phase when about 1/4 of the calves had arrived in the room a higher speed of transmission was gained and continued as such throughout the rest of the study. In this latter phase the spread to susceptibles was independent of their positions relative to those calves demonstrated or suspected to be infectious. This included the spread over the passages separating the pen rows facing each other and even transfers from one side of the room to the other. Significantly, the earlier experimental study and the first phase of the present study differs from the latter part of the present study as regards in the number of high titered excretor calves in the study

room. Because of the great number of excretor calves the chances and degree of exposure for the susceptibles were in all likelihood considerably greater in the latter phase of the present study. This view is supported by the detected difference in the prepositive periods between the early and latter phases of the present study.

The results clearly suggest a rather effective air-borne transmission of M. dispar to have occurred. In the earlier experimental study air-borne transmission over short distances was shown to occur, although such transfer was delayed as compared to that occurring at exposures through direct contact (*Tanskanen* 1987b). Transmission through indirect means, via utensils or via hands or clothes of persons handling the animals, had been meticulously avoided in that study. This condition in that case strongly supported the conclusion of an air-borne mode of transmission to have occurred and, more generally, being both a possible and operational mode for the transmission of this mycoplasma. A probable air-borne spread over short distances has also been reported for other mycoplasma infections (*Bell & Wheeler* 1970, *Hudson* 1971, *Hill* 1972).

It seems less likely that the present study a vehicle transmission, instead of an air-borne transmission, would have occurred in cases of transfers with no direct physical contact between susceptibles and infectives. Firstly, the transfers during the latter part of the survey occurred without any observable variation in the prepositive periods, a pattern less expected of vehicle than air-borne transmission. Secondly, no special effect by any known vehicle factors was noticed. The rather normal management practices in the present study did not as such exclude vehicle transmission as a possible mode. No association of transfer, however, was revealed with the sampling order proceeding from infective calves to susceptibles that was practiced during part of the survey; this order conceivably increased the chances of indirect infective contact between calves through hands and clothes – if such a type of contact were operative. The practices of feeding and cleaning were carried out by rows of pens and opportunities for spread within each row thus appeared larger than those across the rows; yet no indications of corresponding restrictions in transmission were noticed. The experiments with or observations of other mycoplasma species concerning vehicle transmission have generally given negative results (*Hudson* 1971, *Goodwin* 1972).

In the conditions of the previous field study (*Tanskanen* 1987c) an occurrence of air-borne spread, with the exception of a short-range droplet contact, seemed less likely due to the fact that 2 of the susceptibles on the farm surveyed became infected after a long delay. As the susceptible calves, other than the above mentioned 2, in that study possibly could have caught, and a proportion of them evidently caught, the infection already in the transport lorry, the data on the general issue of any air-borne transmission on the farm itself remained, however, equivocal. If the evidence provided by the delayed infection of the 2 calves is taken to be significant of a difference in transmission rate, and possibly mode, between the 2 farms, the explanation might well be sought in the fact that the ventilation system in the setting of the present study, distributing the air effectively into the various parts of the room, was more likely to favour air-borne transmission more than the merely natural ventilation functioning in the calfhouse of the previous field study.

Absence of M. dispar infection in the experimental calves subjected to exposure in the experimental room is a rather safe assump-

tion, because (i) none of the adult cows sampled were found to harbour the infection, and, in general, isolations of M. dispar from adult cattle have not been reported in the relevant literature and (ii) after birth, before and during the transfer to the experimental room, no contact between infected calves and study calves was allowed.

Negativity in the present study of all adult cows sampled indicates, in general, an age restriction for the epidemiology of M. dispar infection and, specifically, the non-transmission of infection vertically from dam to the offspring. A gradual decrease in the colonisation and assumed elimination of infection in experimental calves after a duration of 13 to 17 months, as suggested in results recorded by the present author (*Tanskanen* 1983), are earlier evidence to the specificity of M. dispar infection to calves and young stock. Because of this epidemiology of M. dispar seems to differ from that of several other mycoplasma species (*Lemcke* 1961, *Hudson* 1971, *Whittlestone* 1973, *Jones et al.* 1979, *Pfutzner & Schimmel* 1985).

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References

Bell DP, Wheeler SM: Susceptibility of caesarian-derived rats to natural infection with chronic respiratory disease. Lab. Anim. 1970, *4,* 45–53.

Goodwin RFW: Experiments on the transmissibility of enzootic pneumonia of pigs. Res. Vet. Sci. 1972, *13,* 257–261.

Gourlay RN, Leach RH: A new mycoplasma species isolated from pneumonic lungs of calves (Mycoplasma dispar sp. nov.). J. med. Microbiol. 1970, *3,* 111–123.

Hill A: Transmission of Mycoplasma pulmonis between rats. Lab. Anim. 1972, *6,* 331–336.

Hudson JR: Contagious bovine pleuropneumonia. FAO Agricultural Studies, 1971, no. 86, FAO, Rome.

Jones GE, Buxton D, Harker D: Respiratory infections in housed sheep, with particular reference to mycoplasmas. Vet. Microbiol. 1979, *4,* 47–59.

Lemcke, RM: Association of PPLO infection and antibody response in rats and mice. J. Hyg. (Camb.) 1961, *59,* 401–412.

Pfutzner H, Schimmel D: Mycoplasma (M.)-bovis-Nachweise bei Nachkommen von an M. bovis-Mastitis erkrankten Kuhen und ihre epizootiologische Bedeutung. (Demonstration of Mycoplasma (M.) bovis in descendants of cows affected with M. bovis mastitis and its epidemiological significance). Zbl. Vet. Med. B. 1985, *32,* 265–279.

Reed LJ, Muench H: A simple method of estimating fifty per cent endpoints. Amer. J. Hyg. 1938, *27,* 493–497.

Tanskanen R: Longstanding colonisation of the respiratory tract of calves by Mycoplasma dispar. Acta vet. scand. 1983, *24,* 334–336.

Tanskanen R: Colonisation pattern of the respiratory tract of calves by Mycoplasma dispar. Acta vet. scand. 1984, *25,* 577–592.

Tanskanen R: The prevalence and level of colonisation by Mycoplasma dispar and other mycoplasmas on calf rearing farms. Acta vet. scand. 1987a, *28,* 209–226.

Tanskanen R: Transmissibility of Mycoplasma dispar under experimental conditions. Acta vet. scand. 1987b, *28,* 227–240.

Tanskanen R: Transmission of Mycoplasma dispar among calves collected and reared for beef production. Acta vet. scand. 1987c, *28,* 241–248.

Whittlestone P: Enzootic pneumonia of pigs (EPP). Adv. Vet. Sci. Comp. Med. 1973, *17,* 1–55.

Sammendrag

Spridning av Mycoplasma dispar hos en serie nyfödda kalvar i en mjölkbesättning.

Spridning av Mycoplasma dispar bland 33 nyfödda kalvar som i födelseordning flyttades till egna boxar i ett separat kalvuppfödningsrum undersöktes under en 50 dagars period. Den första kalven infekterades med avsikt. Prov från näs别slemhinnan togs varje vecka inalles 9 gånger.

Tjugoåtta emottagliga djur (uppgifterna för 4 kalvar lämnades bort p. g. a. försummelser vid provtagningen) visade sig vara positiva efter i medeltal 8.6 dagar (varitionen 5 till 15 dagar) räknat från utsättandet för smittan. Bland de 7 första (kalvarna 2–8) och de senare 21 (kalvarna 9–10 och 15–33) emottagliga djuren fastställdes infektionen efter i medeltal 12.1 och 7.4 dagar i angiven ordning. Den uppmätta tiden bestod av 3 delar: den egentliga spridningstiden, latenstiden och den tekniska förseningen vid isoleringen, som en följd av tidsintervallen mellan provtagningarna. Avvikelsen inom de två första delarna, i synnerhet spridningstiden, förklarar tydligt skillnaden mellan undersökningens ovannämnda två grupper. Ett samband med tätheten av infekterade kalvar var uppenbar. Den sista delen av tidsmåttet fördelades på måfå och dess förlängande effekt på värdena uppskattades till mellan 2 och 3 dagar. Genom att subtrahera ovannämnda värde uppskattades medeltalet för tiden som förgick den påvisade kolonisationen i den senare delen av undersökningen till ca. 4 till 5 dagar. Med Reed-Muenchs uträkning blev värdet 4.7 dagar. Nuvarande kunskap om latenstidens längd visar att fördröjningen vid spridningen, d.v.s. den egentliga spridningstiden i undersökningens senare del, knappast överskred några timmar. Därmed kan största delen av den i medeltal 4 dagar långa intervallen betraktas som latensperiod. En ytterligare slutsats var att den huvudsakliga spridningsvägen är aerogen.

Prov som togs av fyrtiofem kor efter förlossningen inkluderande mödrarna till de flesta kalvarna i undersökningen, var alla negativa med avseende på M. dispar, M. bovirhinis och Acholeplasma laidlawii isolerades från 8 och 3 kor i angiven ordning.

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