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LISTERIA MONOCYTOGENES EXCRETION AND HUMORAL IMMUNITY IN GOATS IN A HERD WITH OUTBREAKS OF LISTERIOSIS AND IN A HEALTHY HERD*

By

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LØKEN, T., E. ASPØY and H. GRØNSTØL: *Listeria monocytogenes excretion and humoral immunity in goats in a herd with outbreaks of listeriosis and in a healthy herd.* Acta vet. scand. 1982, *23*, 392—399. — In a herd of 65 goats with outbreaks of listeriosis (Herd A) blood, faeces and milk were collected just after the outbreaks, about 1 month later and at delivery about 4 months thereafter. Faeces and milk were examined bacteriologically and blood and milk serologically for Listeria monocytogenes (Lm), and the results were compared with those of 2 similar samplings in a healthy herd (Herd B).

In Herd A Lm was isolated from faeces in 5 of 14 septicaemic does and in 6 of 48 other animals on the first sampling, and in 4 and 1 animals respectively, on the subsequent 2 samplings. In milk Lm was demonstrated just after the outbreaks only, viz. in 3 of 12 septicaemic does and in 16 of the other 32 examined. Four does excreted Lm in both faeces and milk on this date. In Herd B Lm was demonstrated only at delivery, i.e. from 10 of 43 animals. Most of the isolates belonged to serotype 1.

Reciprocal geometrical mean titres (GMT) of antibodies in sera from the septicaemic group decreased from 236 to 140 and 136 respectively on the subsequent samplings, whereas GMT of the encephalitic animals and of the remainder of Herd A increased from about 20 to about 100 at delivery. GMT of Herd B increased toward delivery from 23 to 39, with largest increase for the does. GMT in whey were ≤ 18 for all groups.

Listeria monocytogenes; Listeria excretion; goats; humoral immunity.

Outbreak of listeric septicaemia followed by encephalitis in a herd of non pregnant goats has been described by *Løken & Grønstøl* (1982). In the present paper a comparison is made

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between that herd and a healthy control herd as to the excretion of Listeria monocytogenes (Lm) in faeces and milk and level of haemagglutinating antibodies against Lm in sera and whey.

MATERIALS AND METHODS

Affected herd (Herd A)

The herd consisted of 50 dairy goats and 17 kids about 8 months old. Two does died in the course of the outbreaks and 11 were culled during the investigation period. The herd was housed from Sept. 18th when most of the does became ill with symptoms which lasted for 1—2 days. During the following 3 weeks 17 does were more severely affected with fever, anorexia and hypogalactia and some had diarrhoea. The clinical findings indicated listeric septicaemia. Seven cases of listeric encephalitis occurred during the period from Oct. 7th to Oct. 10th. All animals except 2, which died, recovered within October. Most of the animals were mated in this month. The herd was vaccinated with a polyvalent Clostridium vaccine (Trivexin®, The Wellcome Foundation Ltd., London) and treated against gastrointestinal parasites before delivery. Further details about management and herd history are described elsewhere (*Løken & Grønstøl* 1982).

Healthy herd (Herd B)

This herd consisted of 30 dairy goats and 13 kids about 10 months old. The goats were of the same breed as those in Herd A. The animals were housed from Nov. 25th, but were given grass silage from the first days of October. All animals were mated in September. One case of listeric encephalitis had occurred during the previous winter, but the general health condition in the herd had been good. Management was similar to that of Herd A.

Bacteriological examination

Faeces and milk were collected at intervals as indicated in Table 1, and were examined for Lm as described by *Grønstøl* (1979a). The propolis-agar (PA, *Grønstøl & Aspøy* 1977) used as selective medium for the isolation of Lm was modified in this examination. Two ml polysorbatum 20 (Tween 20, Chemische Fabrik Hefti, Zürich) was added to each 3.2 ml of alcohol/crude propolis mixture, and after shaking the other components were added and suspended in 1000 ml of the agar.

Serological examination

Blood samples were collected at dates indicated in Table 2. Milk was collected on Oct. 23rd in Herd A and on Nov. 14th in both herds. All samples of serum and whey were stored at —20°C until examined for antibodies against Lm by an indirect haemagglutination method (IHA) as described by *Grønstøl* (1979 a).

RESULTS

Bacteriological examination

H e r d A. Lm was isolated from several samples of faeces and milk shortly after the outbreak (Table 1). The number of faecal samples containing Lm then declined, and at delivery only 1 of 50 was positive. In milk the bacterium was found shortly after the outbreak only. Lm was isolated from a total number of 35 samples. In 27 of these serotype 1 was demonstrated, in

T a b l e 1. Isolation of Listeria monocytogenes (Lm) from faeces and milk in goats in a herd with outbreaks of listeriosis (A) and in a healthy herd (B). The last samples were collected from each goat on the day of delivery, which occurred mostly within March in Herd A and January in Herd B.

Animals	Faeces						Milk					
	Oct. 12-23		Nov. 14		Delivery		Oct. 23		Nov. 14		Delivery	
	n	pos.	n	pos.	n	pos.	n	pos.	n	pos.	n	pos.
Herd A												
Goats without severe symptoms*	42	6	41	1	38	1	28	14	28	0	38	0
Goats with encephalitis*	6	0	5	2	4	0	4	2	4	0	4	0
Goats with septicaemia	14	5	16	1	8	0	12	3	12	0	8	0
Total	62	11	62	4	50	1	44	19	44	0	50	0
Herd B												
Total number of goats*	—	—	43	0	43	9	—	—	30	0	43	5

n: Number of samples examined.
pos.: „ „ „ positive for Lm.
—: Not examined.
*: Pregnant animals less than 1 year that did not yield milk before delivery are included.

6 samples serotype 4 and in 4 samples both serotypes were found. Seven animals excreted both serotypes in either milk or faeces.

H e r d B. In this flock Lm was demonstrated in a total of 10 animals at delivery (Table 1). Four of these excreted the bacterium in both faeces and milk. Of the 14 isolates 9 belonged to serotype 1 and 3 belonged to serotype 4. Two animals excreted Lm of both serotypes.

Serological examination

H e r d A. The reciprocal geometrical mean titres (GMT) of antibodies to Lm in sera from various groups are recorded in Table 2. On the first 2 sampling dates the septicaemic does had significantly higher GMT than both the encephalitis and the remaining animals ($P < 0.001$). At delivery the titres of the 2 groups without septicaemia had increased and nearly reached the GMT of the septicaemic does whose titres had declined. GMT of the 17 kids at delivery was higher than that of the 28 does without septicaemia, i.e. 120 and 91 respectively.

T a b l e 2. Reciprocal geometrical mean titres (GMT) of antibodies to Listeria monocytogenes in sera from groups of goats during pregnancy, in a herd with outbreaks of listeriosis (A) from Sept. 18 to Oct. 10 and in a healthy herd (B).

Animals	Oct. 12-23		Nov. 14		Jan. 17*		March 21**	
	n	GMT	n	GMT	n	GMT	n	GMT
Herd A								
Goats without severe symptoms	43	19	43	18	—	—	40	102
Goats with encephalitis	6	25	5	13	—	—	5	92
Goats with septicaemia	16	236	16	140	—	—	9	136
Herd B								
Total number of goats	—	—	43	23	43	39	—	—

n: Number of samples examined.
—: Not examined.
*: Mean delivery date in Herd B.
**: „ „ „ „ Herd A.

GMT in whey of all groups ranged between < 10 and 18, the latter recorded in the septicaemic does just after the outbreaks.

H e r d B. GMT in sera increased moderately toward delivery (Table 2). At that time GMT of the does was significantly higher than that of the kids ($P < 0.05$).

In whey GMT was 11 for both does and kids.

DISCUSSION

The management and breed in both herds in this investigation were quite similar. However, the delivery occurred about 2 months later in Herd A than in Herd B. The animals were consequently in different stages of the gestation period when samples were collected in November. This might have influenced the isolation patterns, but probably not to any great extent since samples were taken during the first 3 months of pregnancy in both flocks.

In Herd A there was no significant difference in excretion pattern between the septicaemic group and the other animals.

Just after the outbreaks Lm was isolated from the milk in 19 of 44 does (43 %). This corresponds with observations in experimentally infected sheep. *Ivanov et al.* (1964) thus found the greatest number of excretors in milk during the period with illness.

The high excretion rate in milk from goats without severe symptoms indicates bacteraemia in a large proportion of the animals. The reduction in excretors during the first month after the outbreaks, with virtually none at delivery, follows the same pattern as described by *Grønstøl* (1979 b) in a sheep flock with outbreak of abortion.

In the healthy herd Lm was demonstrated neither in faeces nor in milk during pregnancy. However, at delivery Lm was isolated from faeces or milk in 10 of the 43 (23 %) animals. The isolation pattern in this herd corresponds with that found in sheep by *Grønstøl* (1979 a, 1980), and indicates that also healthy goats may be carriers of Lm and excrete the bacteria in periods of stress.

As goat milk is used for consumption or for dairy produce, the excretion and survival of Lm in milk may be of importance in food hygiene. In dairies in this country goat milk is pasteurized (73°C for 15 s), a process which usually inactivates this

bacterium. However, in heavily contaminated samples some organisms may survive this treatment (*Buxton & Fraser* 1977).

Most of the isolates in both herds belonged to serotype 1. Some animals excreted Lm of both serotype 1 and 4. Any obvious association between serotype and course of the disease was not observed. *Kummeneje* (1975) found serotypes 4 and 1 of equal importance in encephalitis in goats, whereas abortions almost exclusively were associated with serotype 1.

The high GMT of the septicaemic group on the first 2 sampling dates was probably caused by a generalized infection with Lm, which triggered off a marked immunological response. In another goat herd with outbreak of abortion, GMT was 305 in sera from 24 affected does (*Grønstøl & Løken* 1979 unpublished). GMT of the same order was found in a sheep flock with outbreak of abortions (*Grønstøl* 1979 b).

With the exception of the septicaemic group, the GMT in both Herd A and Herd B corresponded with those found by *Grønstøl* (1979 a, b) in healthy sheep and in sheep with encephalitis. These results clearly demonstrate the usefulness of serological examination in the diagnosis of listeric septicaemia, which in pregnant animals may cause abortion.

The kids in Herd A had higher GMT at delivery than the does without septicaemia, while the kids in Herd B had statistically lower GMT than the does ($P < 0.05$). This is in accordance with observations in sheep (*Grønstøl* 1979 a, b).

The higher GMT of Herd A than of Herd B at delivery may reflect a stronger pressure of Lm infection in Herd A during the investigation period. This pressure was possibly due to the high excretion rate of Lm and consequently to a stronger contamination of the habitats of the animals. Lm is frequently isolated from feed and environment of animals (*Killinger & Mansfield* 1970). The suggested immunosuppressive effect associated with pregnancy (*Grønstøl* 1979 a) was most likely similar in both herds.

GMT in whey was about 10 for both herds and all groups. The individual titres ranged from < 10 to 80. The IHA-test probably does not reflect the true immunity against Lm in whey, as the test mainly records IgM (*Grønstøl* 1979 b). The predominant immunoglobulin in milk of ruminants is IgG (*Tizard* 1977).

The present investigation showed that goats may excrete Lm in faeces and milk during and after outbreaks of listeric infec-

tions, that healthy goats may be carriers of Lm and excrete the bacteria at delivery and probably also in periods with influence from other stress factors, and that the diagnosis listeric septicaemia may be based on serological examination.

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SAMANDRAG

Utskiljing av og humoral immunitet mot Listeria monocytogenes hjå geiter i ein flokk med utbrot av listeriose og i ein frisk flokk.

I ein flokk på 65 geiter med utbrot av listeriose (flokk A), vart det teke prøver av blod, feces og mjølk i tilslutnad til utbrota, omlag 1 månad seinare og ved kjeing 4 månader deretter. Feces og mjølk vart undersøkt bakteriologisk og blod og mjølk serologisk for Listeria monocytogenes (Lm). Resultata vart samanlikna med 2 tilsvarande prøveuttak i ein frisk flokk (flokk B).

Ved første prøveuttak i flokk A vart Lm isolert frå feces hjå 5 av 14 geiter med septikemi og hjå 6 av 48 av dei andre dyra. I dei 2

påfølgjande prøveuttaka vart bakterien isolert hjå høvesvis 4 og 1 geit. I mjølk vart Lm påvist berre i prøver uttekne nær utbrota, då hjå 3 av 12 geiter med septikemi og hjå 16 av 32 andre. 4 geiter skilde ut bakterien både i mjølk og feces. I flokk B vart Lm påvist berre ved kjeing. 10 av 43 dyr skilde ut bakteriar, og 4 både i mjølk og feces. I dei fleste tilfella tilhøyrde bakteriane serotype 1.

Det resiproke geometriske middeltiteret (GMT) av antistoff i sera frå dyr med septikemi avtok frå høvesvis 236 til 140 og 136 ved dei påføygjande prøveuttaka. I same tidsrom auka GMT hjå dyra med encephalitt og hjå dei andre dyra i flokk A frå omlag 20 til omlag 100. GMT for flokk B auka frå 23 til 39, med størst auke hjå vaksne geiter. GMT i mjølk var $\leq$ 18 hjå alle gruppene.

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