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SEROLOGICAL OBSERVATIONS ON CHLAMYDIA PSITTACI INFECTION IN FINNISH DAIRY HERDS

By

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NEUVONEN, ERKKI and ERKKI PYÖRÄLÄ: *Serological observations on Chlamydia psittaci infection in Finnish dairy herds.* Acta vet. scand. 1981, 22, 1—8. — Chlamydial complement fixing antibodies were followed in 5 small Finnish dairy herds. All 53 animals in the herds were bled. In herds A, B, C and D the blood samples were collected in May and in the following autumn after the pasturing period and in herd E over a period of 2 years at about 1½-month intervals. The frequencies of positive reactions among the adults and the calves younger than 1 year did not differ significantly from each other, but there were considerable seasonal variations in the frequencies. The highest frequency was found during springtime and early summer and the lowest in autumn.

bovine chlamydial infection; group specific chlamydial antibodies.

Chlamydia psittaci is known to cause several clinical manifestations of chlamydiosis. In cows and other domestic mammals the most common are enteritis (York & Baker 1951), abortions (Storz *et al.* 1960), arthritis (Storz *et al.* 1966, Kölbl & Psota 1968), pneumonia (Gorbanov 1964) and conjunctivitis (Dymł 1965). A part of the infections are subclinical and generally only detectable by serological methods and isolation of the agent. Latent infections have been described (York & Baker, Storz 1971) and they may be common in subclinical chlamydiosis.

Most of the chlamydial infections in ruminants spread orally (Storz, Storz *et al.* 1971), but airborne respiratory transmission is possible like in other animals (Storz). There is epidemiological evidence that the agent can be spread by flies (Page *et al.* 1975)

and *Chlamydia psittaci* has also been isolated in several species of ticks and fleas (*Giroud et al.* 1958, *Eddie et al.* 1962, *Digregorio & Johnson* 1967, *Eddie et al.* 1969), but final confirmation of the role of arthropods is still missing. In Finland, the frequency of complement fixing antibodies to the group specific chlamydia antigen in cattle sera is significantly higher in the northern part of the country than in the south. Cattle pasturing in the forest in the summertime have a higher antibody frequency than cattle in field pastures (*Neuvonen & Estola* 1974, *Neuvonen* 1976, 1979). No explanation for these differences in antibody frequencies has been found yet. The average prevalence of chlamydial infections. The method most used, the complement fixation 20 % (*Neuvonen & Estola, Neuvonen* 1976), but except for one case of suspected unreported bovine pneumonia, the titers have not been connected with clinical chlamydial diseases.

Serological methods are of limited value in studies on chlamydial infections. The method most used, the complement fixation (CF) test, is incapable of detecting active immunity in calves until they are 1½—2 months old (*Storz et al.* 1968), and only some healthy sheep (*Storz & Thornley* 1966) and cattle (*Rønsholt* 1978) with chlamydial agents in the feces have significant CF titers. CF antibodies are generally detectable only for a short time; they reach maximal titers within 4 weeks of the infections and then decline over a period of a few months (*Storz*).

The object of this work was to follow the titers of CF antibodies in Finnish dairy herds to gain an understanding of the nature of the chlamydial infection.

MATERIALS AND METHODS

Sera

A total of 344 sera were collected from 53 animals in 5 dairy herds (A, B, C, D, E). All the sera were inactivated for ½ h at 56°C, stored at -20°C and then tested at the same time. All animals tested were of the Ayrshire breed and originated from within the herds. Herds A, B, C and D were bled twice, in May and in the autumn (Table 1). In herd E all the animals were bled at intervals of about 1.5 months over a period of 2 years (Tables 2a & b). Six of the 15 animals of the herd were younger than 1 year at the beginning of the serum collection, the others were older than 2 years. The herds were typical Finnish small-scale

dairy herds pasturing throughout the day from June to October. The animals showed no specific signs of clinical chlamydiosis during the investigation. Parallel isolation investigations from the tested animals were not made.

Assay of antibodies

A microtechnique of the direct complement fixation (CF) test was used (Neuvonen & Estola 1974). Two units of antigen and 2 units of hemolysin were used in the test. Titers of 1:16 or higher were considered positive. The antigen used was an ornithosis antigen prepared from the strain P-4 (Behringwerke).

RESULTS

The positive titers were low, 1:16, 1:32, or 1:64. Ninety-six of the 344 samples (27 %) were positive, but the frequency of positive reactions varied significantly among the different sampling dates.

In herd A (Table 1) the frequency was higher at the first sampling than at the second. Two animals lost their positive

Table 1. Titers of chlamydial CF antibodies in pre- and post-pasture serum specimens.

Animal No.	Date of sampling							
	Herd A		Herd B		Herd C		Herd D	
	May 22	Sep 14	May 18	Oct 10	May 18	Aug 28	May 15	Aug 28
1	<1:8	1:8	<1:8	<1:8	1:8	1:8	<i>1:16</i>	<i>1:16</i>
2	<i>1:16</i>	<i>1:16</i>	<1:8	<1:8	<1:8	<1:8	1:8	1:8
3	<i>1:16</i>	<i>1:16</i>	1:32	1:8	<i>1:16</i>	<i>1:16</i>	<1:8	<1:8
4	<1:8	<1:8	<1:8	<i>1:16</i>	<i>1:32</i>	<i>1:32</i>	<1:8	<1:8
5	<i>1:16</i>	1:8	<1:8	<i>1:16</i>	<i>1:16</i>	1:8	<1:8	<1:8
6	<i>1:16</i>	<1:8	<i>1:16</i>	<i>1:16</i>	<1:8	<1:8	<i>1:16</i>	1:8
7			<i>1:16</i>	<1:8	<1:8	<1:8	<1:8	<1:8
8			1:8	<i>1:16</i>	<i>1:16</i>	1:8	<1:8	<1:8
9			<1:8	<1:8			<i>1:16</i>	<1:8
10			<1:8	<1:8			1:8	<i>1:16</i>
11			<1:8	<1:8			<1:8	<1:8
12							<1:8	<1:8
Number of positive/total samples	4/6	2/6	3/11	4/11	4/8	2/8	3/12	2/12

Positive antibody titers are in italics.

Table 2 a. Changes in titers of chlamydial CF antibodies in herd E in 1978.

Animal No.	Date of sampling									Date of calvin
	Jan 30	Mar 14	Apr 14	May 15	Jun 15	Jul 17	Sep 1	Oct 3	Dec 14	
1	1:8	<i>1:16</i>	1:8	1:8	1:8	<1:8	1:8	<1:8	<1:8	Dec 16 - 78
2	<1:8	<1:8	<1:8	1:8	<1:8	<1:8	<1:8	<1:8	<1:8	
3	<1:8	<1:8	<1:8	<i>1:16</i>	<i>1:16</i>	<i>1:16</i>	<i>1:16</i>	1:8	<1:8	Oct 14 - 78
4	<1:8	<1:8	<1:8	<i>1:16</i>	<i>1:16</i>	<i>1:16</i>	1:8	1:8	<1:8	Dec 8 - 77
5	<1:8	<1:8	<1:8	<1:8	1:8	<1:8	<1:8	1:8	<1:8	Jan 20 - 78
6	1:8	1:8	1:8	1:8	1:8	1:8	1:8	1:8	1:8	Aug 1 - 78
7	<i>1:16</i>	<i>1:16</i>	<i>1:16</i>	<i>1:16</i>	<i>1:16</i>	<i>1:16</i>	1:8	1:8	<1:8	Apr 12 - 78
8	<1:8	1:8	1:8	1:8	1:8	<i>1:16</i>	1:8	<i>1:16</i>	<i>1:16</i>	May 21 - 78
9	<1:8	<1:8	<1:8	<1:8	<1:8	<1:8	<1:8	<1:8	1:8	May 8 - 78
x 10	<1:8	1:8	<i>1:16</i>	<i>1:32</i>	<i>1:32</i>	<i>1:16</i>	<i>1:16</i>	<i>1:16</i>	<i>1:16</i>	
x 11	1:8	1:8	<i>1:16</i>	<i>1:32</i>	<i>1:16</i>	<i>1:16</i>	1:8	1:8	<i>1:32</i>	
x 12	<1:8	<1:8	<1:8	<i>1:64</i>	<i>1:64</i>	<i>1:16</i>	<i>1:32</i>	<i>1:16</i>	1:8	
x 13	<1:8	<1:8	<1:8	<i>1:16</i>	<i>1:16</i>	<i>1:16</i>	<i>1:16</i>	1:8	1:8	
x 14	1:8	1:8	1:8	1:8	1:8	<1:8	1:8	1:8	<1:8	
x 15	<1:8	1:8	<1:8	1:8	1:8	1:8	1:8	1:8	<1:8	
Positive reactions	7 %	13 %	20 %	47 %	47 %	53 %	27 %	20 %	20 %	

x = <1 year of age in January 1978.

Positive antibody titers are in italics.

serological reaction during the summer. In herd B the frequency was lower in May than in the autumn; three animals gained and 2 lost positive sero-reaction during the summer and the autumn. In herd C, 2 animals lost and none gained a positive reaction during the summer. In herd D, 1 animal gained and 2 lost positive reaction. In herd E (Table 2 a) in 1978 only 1 of the 15 animals (7 %) was positive in January. The number of positive animals increased very strongly in April (20 %) and May (47 %) and reached a peak in July (53 %). The frequency then decreased to 20 % in October. In January 1979 (Table 2 b) the frequency increased again and reached a peak in April (47 %), after which it decreased towards the end of the year (20 %).

During 1978, 4 of the 6 animals (67 %) younger than 1 year at the beginning of the test and 5 of the 9 adults had a positive reaction (56 %).

Table 2 b. Changes in titers of chlamydial CF antibodies in herd E in 1979.

Animal No.	Date of sampling							Date of calving
	Jan 29	Apr 9	Jun 12	Jul 16	Sep 20	Nov 23	Dec 27	
1	1:8	<i>1:16</i>	<1:8	<1:8	<1:8	<1:8	<1:8	
2	<1:8	<1:8	<1:8	<1:8	<1:8	<1:8	<1:8	
3	<1:8	<1:8	<1:8	<1:8	<1:8	<1:8	<1:8	
4	<1:8	<1:8	<1:8	<1:8	<1:8	<1:8	<1:8	Mar 28 - 79
5	<1:8	<1:8	<1:8	<1:8	<1:8	<1:8	<1:8	Mar 7 - 79
6	1:8	<1:8	<1:8	<1:8	<1:8	<1:8	<1:8	
7	—	—	—	—	—	—	—	
8	<i>1:16</i>	<i>1:16</i>	<i>1:16</i>	<i>1:16</i>	<i>1:16</i>	<i>1:16</i>	<i>1:16</i>	Apr 28 - 79
9	<i>1:64</i>	<i>1:16</i>	<i>1:16</i>	<i>1:16</i>	<i>1:16</i>	<i>1:16</i>	<i>1:16</i>	Apr 20 - 79
x 10	<i>1:16</i>	<i>1:16</i>	<i>1:32</i>	<i>1:16</i>	<i>1:16</i>	<i>1:16</i>	<i>1:32</i>	Oct 24 - 79
x 11	<i>1:16</i>	<i>1:16</i>	<i>1:16</i>	<i>1:16</i>	1:8	1:8	1:8	Oct 11 - 79
x 12	<1:8	<1:8	<1:8	<1:8	<1:8	<1:8	<1:8	Dec 11 - 79
x 13	1:8	<1:8	<1:8	<1:8	<1:8	<1:8	<1:8	
x 14	1:8	<1:8	<1:8	<1:8	<1:8	<1:8	<1:8	Jul 15 - 79
x 15	<i>1:16</i>	<i>1:16</i>	<i>1:16</i>	<i>1:16</i>	<1:8	<1:8	<1:8	
16	<i>1:32</i>	<i>1:16</i>	<i>1:16</i>	<1:8	<1:8	<1:8	<1:8	
Positive reactions	40 %	47 %	40 %	33 %	20 %	20 %	20 %	

x = <1 year of age in January 1978.

Postitive antibody titers are in italics.

DISCUSSION

In Finland, as in the other Nordic countries, the low number of clinical chlamydial manifestations in domestic animals in relation to the rather high prevalence of complement fixing antibody show that the strains of the agent are weakly pathogenic (Friis 1967, Neuvonen & Estola 1974). Most of the clinical cases in the country in ruminants have been respiratory and intestinal forms. There have been no confirmed abortion cases. Herd E (Tables 2a & b) seemed to be continuously contaminated by Chlamydia during the investigation. Twelve out of 16 animals gave positive antibody reactions. Since it is known that not all infected animals react by producing CF antibodies (Storz & Thornley 1966, Rønsholt 1977), probably almost every animal in the herd had been in contact with the organism. During 1978, the percentage of reacting animals was about the same among adults and calves. This observation does not agree with earlier demonstrations of a higher antibody content in young animals than in

adults (*Rønsholt* 1977). The small size of the material may have influenced the result.

Among the adults the positive titers were 1:16, while among the calves only 1 of the 4 positive animals had a titer of 1:16, 2 had a titer of 1:32 and 1 had a titer of 1:64. This supports the assertion that calves are more susceptible to chlamydia than adult animals (*Rønsholt* 1977, 1978). The duration of the positive antibody reaction varied considerably. The titers disappeared in most of the animals within a few months. Animal No. 10 seemed to be positive for almost 2 years. Such a long lasting reaction may mean a persistent infection which has been reported to occur in the bovine intestine (*York & Baker* 1951).

Animals Nos. 8 & 11 had recurrent antibody reactions. The explanation may be a new infection, but reactivation of an old infection is also possible because the latent nature of *Chlamydia psittaci* in cattle has been well known for a long time (*York & Baker, Storz* 1971).

The antibody frequencies in herd E varied considerably during the investigation. In the first year the rate was highest in July, but the strong increase in the frequency in April and May indicates a new wave of infection in the herd in the spring. In the following year the frequency again increased strongly in the spring, although 2 months earlier. In both years the frequencies declined towards the end of the year. It is possible that in summer, decreasing contacts between the animals do not advance the spread of the agent, and when indoor feeding starts, their physical condition is good enough to withstand most of the chlamydial infections. The strong rise in the titers during winter and spring might be due to close contact between animals and their lowered physical condition during the long indoor feeding period. A Polish investigation (*Sadowski et al.* 1973) gives partly similar results, with the highest frequency occurring in the winter months and the lowest in spring and summer, but it is difficult to draw any conclusions from this because of the different farming conditions. Calving stress does not correlate with the increase in antibodies because only 3 out of 16 calving titers increased within 2 months of calving.

In the double serum investigation (Table 1) the decrease in the antibody frequencies in 3 out of 4 herds during pasturing time supports findings for herd E, viz. that spread of *Chlamydia psittaci* in small-scale dairy husbandry tends to be lower during summer conditions.

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SAMMANFATTNING

Serologiska observationer över Chlamydia psittaci infektioner i mjölk producerande besättningar i Finland.

Komplementbindade antikroppar av Chlamydia psittaci följdes i fem finska mjölkproducerande besättningar. Alla 53 djur i besättningarna testades. I besättningarna A, B, C och D samlades blodproven i maj och följande höst efter betesperioden och i besättningen E under två år med ungefär 1½ månaders intervaller. Frekvensen av antikroppar bland de fullvuxna djuren och kalvar yngre än ett år skiljde sig inte signifikant från varandra men betydliga säsong-variationer konstaterades. Frekvensen var högst om våren och i början av sommaren och lägst om hösten.

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