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STUDIES ON THE OCCURRENCE OF PEPTOCOCCUS INDOLICUS AND CORYNEBACTERIUM PYOGENES IN APPARENTLY HEALTHY CATTLE

By

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HØI SØRENSEN, GUNNER: *Studies on the occurrence of Peptococcus indolicus and Corynebacterium pyogenes in apparently healthy cattle.* Acta vet. scand. 1976, 17, 15—24. — A total of 1050 samples from apparently healthy cattle were examined bacteriologically with special regard to *Pc. indolicus* and *Cb. pyogenes*.

Pc. indolicus was found in 58 % of 130 samples from tonsils (slaughterhouse material), in 23 % of 620 samples from the vagina of cows, in 22 % of 100 samples from the vagina of calves and heifers, in 5 % of 100 samples from the conjunctival sac of cows, and in 10 % of 100 samples from the nasal cavity of cows (Table 1). *Cb. pyogenes* was found in 51 %, 17 %, 19 %, 8 %, and 6 %, respectively. Both organisms were found in each of 9 herds examined, though with varying frequency (Tables 2, 3, and 4).

Altogether *Pc. indolicus* was found in 254 (24 %) and *Cb. pyogenes* in 205 (20 %) of the samples examined (Table 1). In 127 samples both organisms were present. Eleven of the strains of *Pc. indolicus* were β -hemolytic, the rest non-hemolytic.

By gel diffusion analysis the strains of *Pc. indolicus* as well as those of *Cb. pyogenes* could be identified with strains originating from pathological conditions in cattle. With Serotype B occurring most frequently, usually two or three different types of *Pc. indolicus* were found in each of the herds examined (Tables 5, 6, and 7).

The investigation has shown that *Pc. indolicus* is widespread among healthy cattle, and given evidence to suggest that *Pc. indolicus* and *Cb. pyogenes* are natural cohabitants.

Peptococcus indolicus; *Corynebacterium pyogenes*; healthy cattle; vagina; tonsils; conjunctiva; nasal cavity.

Peptococcus indolicus was first described by *Christiansen* in 1934 under the name *Micrococcus indolicus*. In accordance with present-day nomenclature the name *Peptococcus indolicus* has been suggested by *Høi Sørensen* (1974 a). Antigenically as well

as biochemically *Pc. indolicus* has been found to be a well defined species, divisible in a number of precipitin-types (Høi Sørensen 1973). It has mainly been demonstrated in summer mastitis secretions, typically occurring together with *Corynebacterium pyogenes* and/or other bacteria (Leth Jørgensen 1937, 1966, Stuart *et al.* 1951, Cornelisse *et al.* 1970, Høi Sørensen 1974 b), but it has been isolated from other conditions in cattle as well (Stuart *et al.* 1951, Cornelisse *et al.* 1970, Høi Sørensen 1974 b), found in the interdigital skin and in the vagina of a few apparently healthy cows (Høi Sørensen 1973). More detailed information about its spread among healthy cattle does not seem to be available.

Cb. pyogenes is considered to be responsible for a variety of suppurative processes in cattle, and such "pyogenes-infections" may cause great economic losses. As is well known, however, *Cb. pyogenes* often occurs together with other organisms, and in summer mastitis it has been shown experimentally that *Pc. indolicus* as well as *Cb. pyogenes* is of etiological significance (Stuart *et al.*, Høi Sørensen 1972).

Reports on the occurrence of *Cb. pyogenes* in healthy cattle are numerous; for example it has been demonstrated in the vagina by Francis (1941), in tonsils by Francis and Grupe (1963), and in the conjunctival sac and the nasal cavity by Skovgaard (1968).

The present investigation was carried out in order to elucidate the spread of *Pc. indolicus* among healthy cattle, with special reference to its possible occurrence together with *Cb. pyogenes*. Samples were therefore collected from sources known often to harbour this latter organism.

MATERIAL AND METHODS

A total of 1050 samples from clinically healthy cattle were examined. The samples were taken with sterile cotton swabs from the following sites:

The vagina. Six hundred and twenty samples were taken from cows in 9 herds, mainly of SDM breed (3 samplings at monthly intervals) and 100 from calves and heifers, aged 2 to 18 months, in 5 of these 9 herds (1 sampling). The samples were taken 5—10 cm inside the vagina.

The conjunctival sac and the nasal cavity. One sample from

each of these sites were taken from 100 cows in 4 of the above-mentioned 9 herds. Samples were taken from both sites in one operation.

Tonsils (slaughter-house material). One hundred samples were taken from tonsils of cows (pooled material from the two tonsils of each cow), and 30 from tonsils of 15 young bulls of ab. 300 kg body weight (1 sample from each tonsil). The tonsils were transported to the laboratory in sterile plastic bags. After opening of the tonsillar sinus and removal of the mucous content with a glowing steel spatula, the content of a crypt was pressed out and collected with a swab.

Cultures on blood-agar plates were made from the samples within a few hours after they had been collected. The plates were incubated anaerobically at 37°C for 48—72 hrs. in “B T L AN-AEROBIC JARS” with hydrogen and 10 % carbon dioxide added.

Pc. indolicus was identified on biochemical and serological criteria (gel diffusion analysis) as described by *Høi Sørensen* (1973), *Cb. pyogenes* by gel diffusion analysis (*Høi Sørensen* 1974 c).

RESULTS

Pc. indolicus was isolated from 254 and *Cb. pyogenes* from 205 of the 1050 samples examined. In 127 samples both organisms were present, and both were demonstrated in all of the 9 herds and in all of the 4 sites comprised by the study (Table 1). Eleven

Table 1. Bacteriological examination of samples from the vagina, the conjunctival sac, the nasal cavity, and tonsils of apparently healthy cattle. (The total material studied).

Sites examined	Number of samples	Positive			% positive	
		total		<i>Pc. ind.</i> + <i>Cb. pyog.</i> (in same sample)	<i>Pc. ind.</i>	<i>Cb. pyog.</i>
		<i>Pc. ind.</i>	<i>Cb. pyog.</i>			
Vagina, cows	620	142	106	71	23	17
Vagina, calves and heifers	100	22	19	11	22	19
Conjunctival sac, cows	100	5	8	2	5	8
Nasal cavity, cows	100	10	6	0	10	6
Tonsils, cows and young bulls*	130	75	66	43	58	51
Total	1050	254	205	127	24	20

* Of 15 sets of tonsils from young bulls, 11 (73 %) gave growth of *Pc. indolicus* and 13 (87 %) of *Cb. pyogenes*.

strains of *Pc. indolicus* (9 of vaginal origin, all from cows in Herd VIII, and 2 originating from tonsils of cows) were β -hemolytic, whereas the rest of the *Pc. indolicus* strains were non-hemolytic.

The positive findings are listed in Tables 1 (the total material studied), 2 and 3 (samples from, respectively, the vagina of cows and the vagina of calves and heifers), and 4 (samples from the conjunctival sac and the nasal cavity of cows).

Table 2. The occurrence of *Peptococcus indolicus* and *Corynebacterium pyogenes* in the vagina of apparently healthy cows in 9 herds. (Three samplings at approximately monthly intervals. Cows not represented at all samplings not included).

Herd	Number of cows	Positive at 1, 2, or 3 samplings			
		total		%	
		Pc. ind.	Cb. pyog.	Pc. ind.	Cb. pyog.
I	22	12	9	55	41
II	18	5	3	28	17
III	10	2	1	20	10
IV	19	11	9	58	47
V	36	16	10	44	28
VI	24	17	14	71	58
VII	19	8	13	42	68
VIII	13	10	8	77	62
IX	31	15	14	48	45
Total	192	96	81	50	42

Table 3. The occurrence of *Peptococcus indolicus* and *Corynebacterium pyogenes* in the vagina of apparently healthy calves and heifers. (One sampling).

Herd	Number of animals	Positive		
		total		Pc. ind. + Cb. pyog. (in same sample)
		Pc. ind.	Cb. pyog.	
I	30	4	2	1
II	9	0	0	0
III	8	0	0	0
IV	21	9	11	7
V	32	9	6	3
Total	100	22	19	11

All of the 254 strains of *Pc. indolicus* fulfilled the biochemical criteria of this species, and by gel diffusion analysis they could all be referred to one of the serotypes A, B, C, D, or E, whereas none of them were of Type F (Table 5). The type distribution appeared to be independent of the origin of the strains (Table 5) and in 8 of the 9 herds examined 2 or 3 different types were found (Tables 6 and 7). In 1 herd (Herd III) only 1 type (Type C) was demonstrated. In 30 cows *Pc. indolicus* was demonstrated in more than 1 of the samples collected from the vagina. In 24 of these cases the 2 or 3 strains isolated agreed antigenically, while they differed in 6 cases. In 8 cows *Pc. indolicus* was isolated from the conjunctival sac and/or from the nasal cavity

Table 4. The occurrence of *Peptococcus indolicus* and *Corynebacterium pyogenes* in the conjunctival sac and the nasal cavity of apparently healthy cows in 4 herds. (One sample from the conjunctival sac (left or right) and one from the nasal cavity (left or right) from each animal).

Herd	Number of cows	Positive			
		Conjunct. sac		Nasal cavity	
		Pc. ind.	Cb. pyog.	Pc. ind.	Cb. pyog.
I	26	0	0	0	0
VI	26	1	5	1	3
VIII	15	0	0	1	1
IX	33	4	3	8	2
Total	100	5	8	10	6

Table 5. Serotyping of *Peptococcus indolicus*. (The type distribution in the total material studied).

Origin of strains	Number of strains	Serotype					
		A	B	C	D	E	F
Vagina, cows	142	0	56	27	24	35	0
Vagina, calves and heifers	22	0	8	1	4	9	0
Conjunctival sac, cows	5	0	2	2	1	0	0
Nasal cavity, cows	10	0	6	2	0	2	0
Tonsils, cows and young bulls	75	2	29	15	13	16	0
Total	254	2	101	47	42	62	0

besides from the vagina. In 5 of these cases the strains from different sites were of the same serotype, while they were of different types in 3 cases. In 2 cows *Pc. indolicus* was found both in the conjunctival sac and in the nasal cavity; in both cases the strains from the 2 sites were of different serotypes.

By gel diffusion analysis all of the 205 strains of *Cb. pyogenes* were found to agree antigenically with Strain S-2, originating from a summer mastitis secretion.

Table 6. Serotyping of *Peptococcus indolicus*. The type distribution of strains originating from the vagina of apparently healthy cows in 9 herds.

Herd	Number of strains classified as Type			
	B	C	D	E
I	6	0	7	4
II	1	3	0	1
III	0	4	0	0
IV	0	4	4	7
V	22	0	0	3
VI	13	0	10	1
VII	6	0	3	4
VIII*	2	10	0	0
IX	6	6	0	15
Total	56	27	24	35

* Of 9 β -hemolytic strains 1 was of Serotype B and 8 of Type C.

Table 7. Serotyping of *Peptococcus indolicus*. The type distribution of strains originating from the conjunctival sac and the nasal cavity of cows in herds VI, VIII, and IX.

Herd	Origin	Number of strains classified as Type			
		B	C	D	E
VI	Conjunctival sac	1			
	Nasal cavity	1			
VIII	Nasal cavity	1			
IX	Conjunctival sac	1	2		1
	Nasal cavity	4	2		2
Total		8	4	0	3

DISCUSSION

The results of this investigation show that *Pc. indolicus* is widespread among healthy cattle, and that it often occurs together with *Cb. pyogenes* (Table 1). The fact that the distribution of the 2 organisms is similar in all the sites examined may even suggest that they are natural cohabitants. This is of interest, since in summer mastitis secretions they are typically found together (*Leth Jørgensen* 1937, 1966, *Stuart et al.* 1951, *Cornelisse et al.* 1970, *Høi Sørensen* 1974 b) and because it has been demonstrated experimentally that both of them are of etiological significance in that disease (*Stuart et al.*, *Høi Sørensen* 1972).

Pc. indolicus and *Cb. pyogenes* were found remarkably frequent in tonsils (Table 1), and although they often dominated the flora on primary plates, they may well have been overlooked on plates with dense growth of other organisms. The actual carrier rate is therefore probably even higher than the recorded findings would indicate.

The 2 organisms were also found very frequently in the vagina, both in cows and in calves and heifers (Tables 1, 2, and 3), but the incidence of positive findings in the vagina varied from herd to herd.

Pc. indolicus and *Cb. pyogenes* were demonstrated also in samples from the conjunctival sac and the nasal cavity, though at a low frequency compared with the rest of the material studied (Tables 1 and 4).

In a previous report (*Høi Sørensen* 1975) the 9 strains of the β -hemolytic variety of *Pc. indolicus*, which were all of vaginal origin, were described together with an α -hemolytic porcine variety. These 9 β -hemolytic strains originated from 8 cows, which, interestingly enough, all belonged to 1 herd (Herd III). Until now, α -hemolytic strains have not been found in cattle.

Altogether 254 strains of *Pc. indolicus* were isolated from apparently healthy cattle, and all of them were found to agree biochemically as well as serologically with strains from pathological material (cf. *Høi Sørensen* 1973). However, it should be noted that while Type C was the commonest type among strains from pathological material, Type B was predominant among strains from healthy cattle (Table 5).

Considering the high carrier rate it is not surprising that 2 or 3 precipitin types of *Pc. indolicus* were usually found in each herd (Tables 6 and 7). Nor is it surprising that different

types could be demonstrated on different mucous membranes of the same animal, or, occasionally, even on the same mucous membrane.

Till now, Type F has not been isolated from cattle.

By gel diffusion analysis (cf. *Kielstein & Kötsche* 1963, 1966, and *Høi Sørensen* 1974c) the strains of *Cb. pyogenes* originating from healthy cattle were found to agree antigenically with strains originating from pathological material.

CONCLUSION

Pc. indolicus is widespread among apparently healthy cattle. It is often found together with *Cb. pyogenes*, and there is some evidence of the 2 organisms being natural cohabitants.

The hemolytic varieties of *Pc. indolicus* seem to be of limited distribution.

Of the 6 precipitin types of *Pc. indolicus* Type B seems to be most commonly occurring in healthy cattle.

The tonsils serve as an essential habitat for *Pc. indolicus* and *Cb. pyogenes*, but both organisms are commonly occurring also in the vagina, and can be demonstrated in the conjunctival sac and in the nasal cavity as well.

Strains of *Pc. indolicus* and *Cb. pyogenes* originating from healthy cattle have been found to be identical with strains originating from pathological conditions in cattle.

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SAMMENDRAG

Undersøgelser over forekomsten af Peptococcus indolicus og Corynebacterium pyogenes hos tilsyneladende sundt kvæg.

Undersøgelsen omfatter 1050 svaberprøver fra tilsyneladende sundt kvæg. Sekshundredeogtyve prøver var fra vagina af køer i 9 besætninger, 100 fra vagina af kalve og kvier i 5 af de 9 besætninger, 100 fra konjunktivalsæk og 100 fra næseslimhinde af køer i 4 af de 9 besætninger, og 130 var fra tonsiller fra køer og ungtyre (slagtehusmateriale).

Pc. indolicus blev isoleret fra ialt 254 (24 %) og *Cb. pyogenes* fra ialt 205 (20 %) af de undersøgte prøver (tabel 1). I 127 prøver blev

begge bakteriearter påvist. Elleve af de isolerede *Pc. indolicus* stammer var β -hæmolytiske, de øvrige non-hæmolytiske.

Pc. indolicus blev påvist i 58 % af tonsilpodningerne, i 23 % af vaginalpodningerne fra køer, i 22 % af vaginalpodningerne fra kalve og kvier, i 5 % af konjunktivalpodningerne og i 10 % af næsepodningerne (tabel 1). *Cb. pyogenes* blev i de tilsvarende prøver påvist i henholdsvis 51 %, 17 %, 19 %, 8 % og 6 %. Begge bakteriearter blev påvist i alle de undersøgte besætninger, omend med varierende hyppighed (tabel 2, 3 og 4).

Såvel *Pc. indolicus* stammerne som *Cb. pyogenes* stammerne fandtes identiske med stammer isoleret fra patologisk materiale fra kvæg (gel diffusions analyse). Oftest påvistes 2 eller 3 forskellige serotyper af *Pc. indolicus* i hver af de undersøgte besætninger (tabel 6 og 7), og type B var den hyppigst isolerede i det samlede materiale (tabel 5).

Resultaterne viser, at *Pc. indolicus* er almindeligt forekommende hos sundt kvæg, og de tyder endvidere på, at *Pc. indolicus* og *Cb. pyogenes* typisk forekommer sammen.

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