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Occurrence, Isolation and Serotyping of Erysipelothrix Rhusiopathiae in Cattle and Pig Slurry*

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Nørrung V., B. Munch and H. Errebo Larsen: Occurrence, isolation and serotyping of Erysipelothrix rhusiopathiae in cattle and pig slurry. Acta vet. scand. 1987, 28, 9–14. – In a survey on the occurrence of potential pathogenic bacteria in slurry, Erysipelothrix rhusiopathiae (E. rh.) was found in samples from 49 % of 84 cattle herds, 44 % of 32 pig herds, and 39 % of 67 cattle and pig herds.
Among E. rh. isolates from 81 of the herds, 16 different serotypes were distinguished, including 1 serotype not previously described, which was designated serotype 23.
Procedures of isolation and serotyping are presented and discussed, and the serotype distribution within the different herd categories is outlined.

Erysipelothrix rhusiopathiae; cattle slurry;pig slurry; isolation procedure; serotyping.

Introduction

There are few reports on the occurrence of Erysipelothrix rhusiopathiae (E. rh.), the causative organism of swine erysipelas, in slurry. In Australia, *Chandler & Craven* (1980) demonstrated E. rh. in 15 of 40 samples of slurry from 35 pig herds. In Denmark, E. rh. was initially observed in batches of both pig and cattle slurry which were collected for experimental purposes on a limited number of farms (*Larsen & Munch* 1981a). In a later survey on the occurrence of selected pathogens in slurry, E. rh. was isolated from a large proportion of slurry samples from 183 cattle and pig herds (*Larsen & Munch* 1981b).

Antigenic heterogeneity of E. rh. was reported by *Watts* (1941), *Atkinson* (1941), and by *Gledhill* (1945) who demonstrated 2 distinct serological groups by means of agglutination tests. *Heuner* (1958) showed the following types: A_1, A_2, B_1, B_2, and C, and since then several other types have been described (*Murase et al.* 1959, *Kucsera* 1963, *Truszczynski* 1963, *Kucsera* 1964, *Castro et al.* 1970, and *Kucsera* 1971).

At the suggestion of *Kucsera* (1972) a new classification system was adopted. In this system serotypes are designated by Arabic numerals and subtypes by small letters, e.g., 1a, 1b, 2a, 2b. The advantages of this are two: a standardized nomenclature, and unlimited possibilities of adding new types.

Fabian et al. (1973) designated a not earlier described type, serotype 16, and *Wood et al.* (1978) demonstrated 4 new types, i.e., serotypes 17, 18, 19, and 20. *Nørrung* (1979) described 2 new serotypes, serotypes 21 and

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22, among strains isolated by Dr. Alberto I. de Diego, Argentina.
In this paper procedures of isolation and serotyping of E. rh. isolates from cattle and pig slurry are given, and the prevalence of isolations as well as serotype distribution within various herd categories are presented.

Materials and methods

Slurry

From November 1979 through August 1980 slurry samples from 84 cattle herds, 67 cattle and pig herds, and 32 pig herds were collected. The disease status as regards E. rh. infection was unknown for all the herds examined.
The majority of the pig herds were situated in 2 counties only, while the rest of the herds were from all over the country. From each farm, 1 sample was examined for the presence of E. rh. The sample consisted of 1 l of slurry, drawn from a batch of pooled subsamples taken with a specially constructed sampler (*Moore & Downey* 1978) at random sites from the outdoor storage tank.
All samples were chilled during transportation to the laboratory and stored at 4°C until examined 4–7 days after collection.

Isolation procedure

A modification of a selective method described by *Wood* (1965) was used. Ten ml of slurry was propagated at 37°C for 72 to 96 h in 90 ml of nutrient broth (Difco B344) with 10 % horse serum and containing 500 µg kanamycin sulphate, 50 µg neomycin sulphate, and 25 µg vancomycin sulphate per ml, followed by plating on nutrient agar (Difco B232) with 10 % horse serum (NA), and with the same admixture of antibiotics. After incubation at 37°C for 24 to 48 h, representatives of small bluish-white semitransparent colonies, suspect of E. rh., were Gram-stained, and tested for catalase activity and reaction in triple sugar iron agar (Difco B235) with 10 % horse serum (TSI). Isolates of catalase negative, Gram-positive rods (*Seeliger* 1974), which on TSI slants showed more or less blackening stopping abruptly just below the water of syneresis (*Vickers & Bierer* 1958), were presumptively diagnosed as E. rh., and subcultures were stored as stabs in NA at room temperature. Later, fresh stab cultures were submitted to the National Veterinary Laboratory for serotyping.

Preparation of antisera

Type specific antisera were prepared in rabbits by immunization with antigens made from reference strains of E. rh. representing the 22 reported serotypes (*Nørrung* 1979). Antisera were also prepared against isolates which could not be determined by established antisera. Antigens used for immunization of rabbits were prepared as follows: A 24-h beef infusion broth culture of a reference strain or an isolate of unknown serotype was inoculated into 200 ml of beef infusion broth with 10 % horse serum. After incubation at 37°C for 24 h the culture was centrifuged and the cells were washed 3 times with saline containing 0.2 % formaline and the density of the suspension was adjusted to McFarland's tube No. 4.
Each antigen was injected i.v. into rabbits at intervals of 3–4 days in doses successively increasing from 0.5 to 6 ml.
Eight days after the last antigen administration the rabbits were exsanguinated. The sera thus obtained were preserved with merthiolate (1:10,000) and stored at –30°C or, if freeze-dried, at 4°C.

Preparation of antigen

Beef infusion broth with 10 % horse serum was inoculated with a smooth colony from a 48-h-old agar plate culture and incubated

for 48 h at 37°C. Thereafter the culture was centrifuged at 1000×g for 30 min. The organisms were washed 3 times with physiological saline and suspended in distilled water to 1/30 of the original volume. The suspension was autoclaved at 120°C for 1 h and centrifuged at 1000×g for 30 min. The supernatant, i.e., the bacterial extract, was preserved with merthiolate (1:10,000). Bacterial extracts can be kept for a long time at 4°C, but are normally stored at −10°C.

Precipitation tests

Gel precipitation tests were performed on 25 × 225 mm large glass slides. The gel consisted of 1 % agar (Special-Agar-Noble, Difco) in saline with merthiolate (0.01 %) as a preservative.

Approximately 10 ml of melted agar was poured onto each slide. After 10 min wells were made with a gel punch. The wells were 2 mm in diameter, and their centre-to-centre distance was 8 mm. Antigen was placed in the centre well and antisera in the surrounding wells, whereafter the slide was placed in a moist chamber at room temperature. Reading was performed after 24 and 48 h.

Results and discussion

Out of 134 presumptive E. rh. isolates, each representing 1 herd, 22 could not be recultured, mainly due to fungal contamination. Of the remaining 112 isolates, 31 were not verified as E. rh., and further identification was not performed. Such bacteria – probably coryneforms – might be of differential diagnostic significance, when the isolation procedure described is used for slurry examination.

E. rh. was isolated from all herd categories, with a verified prevalence of 49, 44, and 39 %, respectively, in herds of cattle, pigs, and cattle + pigs (Table 1). It also appears, that the actual isolation prevalence within the various categories might have been somewhat higher. To our knowledge the isolation of E. rh. from cattle slurry has not been reported by other authors. For comparison, it can be noted that *Wood & Packer* (1972) detected E. rh. in half the samples of manure collected in 11 pig herds with a history of clinical erysipelas within the last 9 months, and also from one-third of samples from 8 herds in which no clinical outbreaks had been observed for the last 5 years.

Table 1. Prevalence of presumptively diagnosed and serologically verified Erysipelothrix rhusiopathiae (E. rh.) isolations in slurry from Danish herds of cattle and pigs.

Herd category	Composition of samples examined	No. presumptive E. rh.*/No. herds examined	No. E. rh. verified*/ No. presumptive E. rh. examined*	Prevalence of verified E. rh. positive herds: No. herds pos./ No. herds exam.	% pos. herds
Cattle only	Cattle slurry	64/84	41/55	41/84	49
Cattle + pigs	Cattle slurry	21/24	10/17	10/24	42
Cattle + pigs	Cattle slurry + pig urine	11/16	9/10	9/16	56
Cattle + pigs	Cattle slurry + pig slurry	14/27	7/11	7/27	26
Pigs only	Pig slurry	24/32	14/19	14/32	44
Total:		134/183	81/112**	81/183	

* Each isolate represents one herd.

** Presumptive E. rh. isolates from 22 herds not available for final identification and serotyping.

Out of a total of 81 herd isolates 78 could be typed by means of the 22 type sera. Five isolates were found to contain 2 different serotypes, i.e., type 1+8, 2+3, 2+5, 5+9, and 7+12.

Table 2 shows the number of strains belonging to the different serotypes. As will be seen, the serotypes of 3 strains could not be determined, as antigen from these strains did not react with any of the established type antisera. Rabbits were immunized with antigen from these strains, and antisera thus produced were tested with antigen from these 3 strains. The results indicated that the 3 strains belonged to one and the same serotype, a serotype which has not been described earlier (Table 3). The type was designated serotype 23, and strain KS 20A is proposed as reference strain.

Table 2. Serotypes of Erysipelothrix rhusiopathiae isolated from slurry of Danish herds af cattle and pigs.

Serotype	Number of strains* (%)
1	2 (2.3)
2	27 (31.4)
3	0
4	1 (1.2)
5	14 (16.3)
6	7 (8.1)
7	2 (2.3)
8	4 (4.7)
9	2 (2.3)
10	3 (3.5)
11	5 (5.8)
12	7 (8.1)
13	1 (1.2)
14	0
15	0
16	3 (3.5)
17	0
18	0
19	3 (3.5)
20	0
21	0
22	2 (2.3)
Undetermined	3 (3.5)

* Five isolates contained two serotypes (see text).

Table 3. Cross precipitation tests between extracts from 3 undetermined Erysiopelothrix rhusiopathiae strains and antisera raised against these strains.

Antigens/antisera	KS 20A	KS 26A	S 18A
KS 20A	+	+	+
KS 26A	+	+	+
S 18A	+	+	+

The distribution of the serotypes represented within the 81 herd isolates, according to their occurrence in samples from the different herd categories is given in Table 4. It must be borne in mind that the material presented is incomplete, as potential E. rh. strains among the isolates not being available for serotyping are not included. It should also be remembered that 1 isolate only from each of the 81 herds has been serotyped. On account of this and the relative smallness of the total material, no extensive conclusions can be drawn as to e.g., 'host preferences' of the serotypes, or epidemiological significance of their distribution in the material.

Even so, it can be noted that serotype 2 was recovered in all types of slurry examined. It was found comparatively more often in cattle slurry (19 % of samples from altogether 108 herds, and 39 % of altogether 51 E. rh. isolates) than in mixtures of cattle slurry and slurry or urine from pigs, or in pig slurry (both 9 % of samples from 43 and 32 herds, and 25 and 21 % of 16 and 14 isolates, respectively).

Serotype 5 was represented in most types of samples, while the other serotypes were distributed more or less scattered in the material. It can be added that the new serotype

Table 4. Distribution of serotypes represented within 81 isolates of Erysipelothrix rhusiopathiae (E. rh.) from slurry of Danish herds of cattle and pigs.

Herd category	Composition of samples examined	No. E. rh. pos. herds/ No. herds examined*	E. rh. serotypes, No. of isolates within herd categories specified:																			
			1	1+8	2	2+4	2+5	5	5+9	6	7+12	7+14	8	9	10	11	12	13	16	19	22	23
Cattle only	Cattle slurry	41/84	1	1	13	1	1	7	–	5	1	–	–	1	1	3	3	–	1	1	1	–
Cattle + pigs	Cattle slurry	10/24	–	–	5	–	–	1	–	–	–	–	1	–	–	1	1	1	–	–	–	–
Cattle + pigs	Cattle slurry + pig urine	9/16	–	–	2	–	–	2	–	1	–	–	–	–	–	–	–	–	1	2	1	–
Cattle + pigs	Cattle slurry + pig slurry	7/27	–	–	2	–	–	–	–	1	–	–	–	–	1	1	–	–	–	–	–	2
Pigs only	Pig slurry	14/32	–	–	3	–	–	2	1	–	–	1	2	–	1	–	2	–	1	–	–	1
Total		81/183	1	1	25	1	1	12	1	7	1	1	3	1	3	5	6	1	3	3	2	3

* cf. Table 1.
– not represented.

was isolated from samples of cattle + pig slurry from 2 herds in the same county, collected in June 1980, and from a sample from a pig herd in a different county, collected in August 1980. Besides, no evident relationships could be revealed between area or period of slurry collection, and occurrence of certain serotypes in the slurry samples.

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Sammendrag

Forekomst, påvisning og serologisk typebestemmelse af Erysipelothrix rhusiopathiae i kvæg- og svinegylle.

Ved en undersøgelse af gylle fra danske kvæg- og svinebesætninger for muligt indhold af en række potentielt patogene bakterier påvistes Erysipelothrix rhusiopathiae i prøver fra 49 % af 84 kvægbesætninger, 44 % af 32 svinebesætninger og 39 % af 67 kvæg- og svinebesætninger.

Erysipelothrix rhusiopathiae-isolater fra 81 af besætningerne blev underkastet serologisk typebestemmelse, og der fandtes 16 forskellige serotyper, hvoraf en ikke tidligere er beskrevet. Denne blev betegnet serotype 23 med stamme KS 20A som typestamme.

De anvendte metoder til isolation og serotypebestemmelse beskrives og diskuteres, og der gives en oversigt over fordelingen af serotyper de forskellige besætningskategorier imellem.

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