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From the State Veterinary Serum Laboratory, Copenhagen, Denmark.

STUDIES ON ERYSIPELOTHRIX INSIDIOSA S. RHUSIOPATHIAE*)

1. MORPHOLOGY, CULTURAL FEATURES, BIOCHEMICAL REACTIONS AND VIRULENCE

By

V. Nørrung

According to *Pasteur & Thuillier* (1883) E. insidiosa was discovered by Thuillier on March 15, 1882.

By means of inoculation of pigs with broth culture of this organism, *Pasteur & Thuillier* obtained the characteristic clinical picture of swine erysipelas, thus showing that the organisms were the cause of the disease. However, Pasteur stated emphatically that the finding applied to certain breeds of pig only, which observation was later to have great significance in connection with vaccination against the disease.

In trying to date back the first cases in Denmark, *Jensen* wrote in 1892: "The oldest report of the disease originates supposedly from E. Viborg who, in a book on the management of the pig published in 1804, gave a detailed description of 'anthrax' in swine. Though he made no mention of the characteristic redness of the skin, there can be no doubt that the disease referred to was swine erysipelas."

As is known, distinction is made between the following clinical forms: acute swine erysipelas, diamond disease, endocarditis, arthritis, and dry gangrene.

The incubation period in artificial infection with strongly virulent E. insidiosa is 1—3 days. It is difficult to state a definite incubation period in the natural infection.

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It is a fact that many clinically healthy pigs are carriers for shorter or longer periods, and *Connell & Langford* (1953) isolated the organisms from 30 % of 639 apparently healthy pigs. The organisms were cultured from the tonsils in 115 cases; from tonsils and gall bladder in 29; from tonsils, gall bladder, and intestinal canal in 12; from tonsils and intestinal canal in 12; from gall bladder alone in 7; from gall bladder and intestinal canal in 6; and from the intestinal canal alone in 11 cases.

There was great seasonal variation, the majority of the positive cultures being found in the month of July and August.

As mentioned, the disease occurs in different clinical forms. How can the same organism cause so widely varying disease manifestations?

The same question was asked by *Jensen* i 1892. He found 3 possible answers: 1) the route of infection, 2) breed and individual peculiarities, and 3) variations in the virulence of the bacterium.

Having confuted the first 2 possibilities, *Jensen* wrote: "There is therefore nothing left but to assume that it must be the varying degree of virulence of the bacillus, perhaps combined with greater or lesser individual receptivity, that determines the character of the disease and its mild or severe course (diamond disease or swine erysipelas)."

The aim of the present study was to elucidate the problem: do strains of E. insidiosa responsible for chronic forms of swine erysipelas possess any peculiar characteristics as regards morphology, cultural features, biochemistry, or virulence? Later studies will deal with possible differences in antigenic structure, and with the question whether the occurrence of the infection can be diminished or eliminated by vaccination.

MATERIAL

The material originated from the Cooperative Bacon Factory at Sorø*). It consisted of 808 unopened joints from pigs suspected of arthritis because of lameness, swelling of 1 or more joints, or affected (swollen, hyperaemic) regional lymph nodes.

E. insidiosa was isolated from 112 of the 808 specimens, either from joints or regional lymph nodes, or both. Table 1 shows the

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serial numbers, slaughter-house numbers, and supplier numbers of the strains examined.

The joints were either sent in to the laboratory immediately after slaughter, or, more frequently, deep-frozen at the slaughter-house and sent in later.

METHODS

The unopened joint was examined by palpation and, after scorching, the joint cavity was opened by an about 1 cm long incision in the joint capsule. A small amount of synovial fluid was pipetted off and inoculated on 5 % blood agar plates.

The joint was then opened completely, and after recording of possible patho-anatomical changes cultures were made from any area with such changes. If there were no changes, cultures were made from scrapings of the synovial capsule and the joint cartilage.

The plates were incubated at 37°C for 24 to 48 hrs., after which they were examined for suspect colonies by means of transmitted light and a magnifying glass. Subcultures of suspect colonies were made on blood agar plates, which were incubated at 37°C for 24 hrs. Gram-stained smears from subcultures were examined by microscopy, and if gram-positive, slender, short rods were found, 0.5 ml of a suspension of a plate culture was inoculated intraperitoneally into mice.

Heart blood of mice that died within 2—4 days was inoculated on blood plates, which were incubated at 37°C for 24 hrs. From the plate cultures fermentation tubes were inoculated for determination of biochemical activity. These tubes were incubated at 37°C and inspected daily. Positive findings were recorded with a plus sign and a figure indicating the number of days that elapsed before the appearance of the positive reaction.

Two different media were used for examination for hydrogen sulphide. Originally, only $FeCl_2$-gelatine was employed, but this medium was difficult to keep in solid form at moderately elevated temperatures, and was therefore replaced by iron agar. However, when it was found that a number of strains did not show formation of H_2S within 3 weeks, the medium described by *Kemenes & Kucsera* (1967) was adopted. This medium, which is semi-fluid, consists of broth to which is added 1 % glucose, 10 % horse serum, 0.2 % sodium thiosulphate, 0.04 % lead acetate and 0.2 % agar.

Table 1. Biochemical reactions compared to virulence.

Strain no.		serial no./ supplier no.	Arabinose	Xylose	Fructose	Maltose	Trehalose	Mannitol	Sorbitol	Mannose	Dextrose	Lactose	Salicin	Galactose	Glycerol	Iron agar	Semi-fluid H_2S medium	Hyaluronidase	Virulence determination: LD50	Virulence determination: no. of bacteria
1	Knee joint	1539/1989	8	3	2	2					2			2		2	1	140	$10^{-6.0}$	144.0
2	Thigh „	1538/1989		3	2	2	14	6			2	3		2	14	3	1	160	$10^{-6.33}$	70.2
3	Knee „	1577/1989		6	1	1					1	9		6		2	1	40	$10^{-5.83}$	357.2
4	„ „	1578/1989	8	8	8	2					2			8		2	1	70	$10^{-6.17}$	179.6
5	„ „	1055/322		6	2	5					1			2		2	1	60	$10^{-5.27}$	1342.0
6	„ „	164/1244		7	2	2				11	1	4		2		2	1	80	$10^{-6.75}$	62.2
7	„ „	849/1279		1	1	4	4			7	1	1	13	1	6	2	1	70	$10^{-6.66}$	61.3
8	„ „	1941/591		6	4	4					1	1		1		3	1	90	$10^{-7.76}$	42.7
9	„ „	311/331		3	3	3					1	11		11		2	1	40	$10^{-6.64}$	91.8
10	„ „	843/580		5	1	1	3				1		1	3		2	1	120	$10^{-7.5}$	9.0
11	Lymph node	759/479		4	1	1					1			4		3	1	100	$10^{-8.0}$	7.3
12	„ „	2267/763		1	1	1					1	13		5		3	1	110	$10^{-7.17}$	13.9
13	Knee joint	799/413		1	1	1					1	2		2		2	1	80	$10^{-6.5}$	94.9
14	Thigh „	740/1508		5	1	1					1	14		5		2	1	130	$10^{-7.0}$	22.8
15	Knee joint	2427/2018		12	1	4					1	11		12		2	1	120	$10^{-5.5}$	430.0
16	„ „	766/16		11	1	1					1	13		4		5	1	150	$10^{-7.33}$	18.0
17	Thigh „	1186/155		1	1	1					1	5		1		3	1	160	$10^{-5.33}$	1946.0
18	Knee „	716/639	5	1	1	1	1	5	5	4	1	4	4	1		2	1	130	$10^{-7.0}$	18.8
19	„ „	16/409		7	1	6					1			6		2	1	100	$10^{-4.66}$	1378.0
20	Thigh „	88/2465		5	1	2					1	5		5		2	1	80	$10^{-5.5}$	289.9
21	Knee „	743/1486			1	3					1					3	1	110	$10^{-6.5}$	100.0
22	„ „	307/80		8	1	2					1	8		8		2	1	140	$10^{-7.5}$	8.4
23	Lymph node	1718/1532		4	3	3					1	4		4		3	1	100	$10^{-4.43}$	5015.0
24	„ „	953/1608			3	4					1					2	1	160	$10^{-6.76}$	27.1
25	„ „	2299/246		6	5	8					1	3				2	1	40	$10^{-6.66}$	45.5
26	Knee joint	128/655			5						2					2	1	90	$10^{-6.66}$	123.6
27	Thigh „	1639/1628			4	6					2					2	1	60	$10^{-7.5}$	8.6
28	Knee „	965/476			4						2					1	1	120	$10^{-6.66}$	74.4
29	„ „	2390/2221			8	7					1					1	1	60	$10^{-6.0}$	136.7
30	Lymph node	1243/433			5						1			1		3	1	80	$10^{-7.0}$	31.5

31	Lymph node	639/587			5	8				1		8		2	1	90	$10^{-6.76}$	84.8
32	Knee joint	588/1801				8				1				2	1	90	$10^{-7.0}$	22.5
33	„ „	671/366			5	10			6	2				2	1	80	$10^{-5.5}$	499.6
34	„ „	1631/1154			7					10				2	1	70	$10^{-6.5}$	49.3
35	„ „	403/1977			2	12				2				2	1	70	$10^{-7.5}$	5.5
36	„ „	225/607												2	1	320		
37	Lymph node	862		14					11	2	2			2	1	70	$10^{-6.0}$	165.0
38	Knee joint	354/1272				13			3	1				2	1	80	$10^{-4.83}$	2003.0
39	Thigh „	1777/1659		13	10					3				5	1	90	$10^{-5.5}$	67.6
40	Lymph node	575/2197		5	1	2				1	10	3		3	1	130	$10^{-6.66}$	14.1
41	„ „	85/1107		10	8					1		11		3	1	160	$10^{-7.0}$	82.5
42	„ „	522/2063		5	5	4				1				2	1	140	$10^{-6.66}$	38.3
43	Thigh joint	380/1001		4	4	4	13			1				2	1	140	$10^{-7.0}$	33.0
44	Knee „	1452/534	7		4	6				1			5	2	1	70	$10^{-7.5}$	5.4
45	Lymph node	1586/865		12		8				1	4	12		3	1	90	$10^{-6.5}$	14.1
46	„ „	2182/1523			8	4			10	1	12	11		2	1	90	$10^{-5.5}$	160.1
47	„ „	1055/580				12				1				2	1	160	$10^{-7.33}$	13.2
48	Knee joint	1215/1639			4	6			5	2	6	12		3	1	90	$10^{-8.5}$	2.1
49	„ „	1362/1626		12						1	6			3	1	100	$10^{-5.33}$	187.4
50	„ „	2350/688		6	5	14				1	3	13		2	1	100	$10^{-4.17}$	4158.0
51	Lymph node	654/1861			4	5				1	11			2	1	130	$10^{-6.5}$	110.7
52	Knee joint	324/865			4					1	4	7		3	1	120	$10^{-6.77}$	23.1
53	„ „	1446/2188			4	1		4		1	4	7		2	1	130	$10^{-6.34}$	19.2
54	Thigh „	2152/2408		7	1	7				1	7	7		—	1	60	$10^{-6.5}$	51.1
55	Knee „	1432/2408		6	2	6				1	2	2		—	1	50	$10^{-6.6}$	13.8
56	Thigh „	1467/2408		6	2					1	2	2		—	1	50	$10^{-6.3}$	98.5
57	„ „	1338/2408		4	4					1	2	13		2	1	110	$10^{-6.5}$	55.1
58	Knee „	1838/2408			10					6				—	1	150	$10^{-6.5}$	89.5
59	Lymph node	306/2408			4					1		4		—	1	160	$10^{-6.6}$	95.5
60	Knee joint	701/2408			7					5				—	1	160	$10^{-5.63}$	212.9
61	„ „	292/2408			3	3			3	3	3	7		2	1	50	$10^{-6.66}$	91.1
62	„ „	1852/2408				13				5				—	1	100	$10^{-7.0}$	20.0

The figures indicate the number of days before positive reaction occurred.
All strains showed negative reaction in the following media: rhamnose, saccharose, adonitol, dulcitol, inositol, mucate, urea, indole, MR, VP, potassium nitrate and sodium citrate.
Almost all strains gave weak acid production in litmus milk.

Determination of the virulence of E. insidiosa. Beef extract broth, pH 7.4, was inoculated with growth from a 24-hr.-old plate culture. After incubation at 37°C for 24 hrs., serial tenfold dilutions of the resulting culture were made in beef extract broth, and 0.5 ml of each of the dilutions 10^{-1} to 10^{-10} injected i.p. into each of 4 mice for determination of LD50. Viable counts were made on dilutions 10^{-6} to 10^{-8} by inoculation of 0.1 ml of each dilution on each of 5 blood agar plates.

LD50 was calculated according to the method described by *Reed & Muench* (1938).

Virulence determination on pigs was performed by intracutaneous injection of 0.1 ml of a 24-hr.-old broth culture, followed by measurements of temperature night and morning and daily recording of possible local reactions.

Determination of hyaluronidase. The method used was that described by *Ewald* (1957) with bovine synovial fluid as source of hyaluronic acid.

The titre of the hyaluronic acid was determined as the reciprocal value of the highest dilution that, when added dropwise to acid alcohol (96 ml 96 % ethyl alcohol, 4 ml 25 % hydrochloric acid), gives a confluent drop. For determination of hyaluronidase in a strain of E. insidiosa a 24-hr.-old broth culture was adjusted to a density of about 500 mill. organisms per ml, corresponding to no. 2 in Coleman's photo-nephelometric scale.

The culture was centrifuged for 30 min. at 3000 r.p.m. and 0.25 ml of each of a series of dilutions (1:10, 1:20, 1:30, etc. up to 1:160, and 1:320) of the supernatant fluid in physiological saline was mixed with 0.25 ml of a solution of synovial fluid with a hyaluronic acid concentration 3 times that found as endpoint in the above-mentioned titration.

After 1 hr. in water bath at 37°C 0.1 ml congo red (0.5 %) was added, and the tubes were shaken vigorously. After 5 min. at room temperature titration was performed.

The highest concentration of culture fluid in which coagulation would occur when a drop was added to acid alcohol would indicate the hyaluronidase titre limit.

RESULTS AND DISCUSSION

The strains of E. insidiosa examined appeared as gram-positive rods, the length of which varied with the moistness of the medium. On very moist medium, e.g. serum agar, the organisms often formed long threads.

Table 2. Virulence tests on pigs.

Pig no.	Strain no.	Temperature: Inoculated 10 a.m. Dec. 9	Dec. 10	Dec. 11	Dec. 12	Dec. 13	Dec. 14	Dec. 15	Skin reaction 3 days after inoculation
1	36		39.0	39.2	39.2	39.0	39.2	38.5	
		39.1	39.0	38.8	38.8	38.6	38.7	38.5	—
2	42		38.5	38.5	39.7	39.6	39.5		
			38.8	38.6	39.1	39.2	39.3		++
3	55		39.6	40.3	39.8	39.2	38.7	38.8	
		39.0	39.5	40.0	39.9	39.5	39.8	39.0	++
4	57		39.3	39.8	39.6	39.7	39.0	39.0	
		38.9	39.5	40.0	39.2	39.9	39.0	39.2	+
5	58		39.5	40.4	40.5	40.7	38.5	38.9	
		39.0	39.0	40.2	40.1	41.5	39.1	39.0	+++
6	60		38.8	40.1	40.0	40.4	39.8	39.6	
		38.5	39.8	40.1	39.8	40.0	39.8	39.5	++
7	62		40.1	39.0	39.1	40.3	40.4	38.6	
		38.4	40.3	38.8	39.3	40.0	39.6	39.5	++
8	Swedish strain R/9		39.9	39.8	39.8	40.4	40.6	39.5	
		39.5	39.8	39.1	39.0	40.2	39.7	39.0	+++

Morning temperature / Evening temperature

+, ++ and +++ indicate the reaction of the site of injection: slight redness, severe redness, very severe redness and swelling.

Table 1 shows the results of the biochemical tests, the virulence determination, and the viable counts.

Biochemically 60 strains were largely similar, all showing a limited biochemical activity. Two strains were clearly different from the others. One (no. 36) showed the lowest degree of biochemical activity, forming H_2S only. The other (no. 18) showed the highest degree of activity.

All strains formed H_2S in semi-fluid H_2S medium, while 7 showed no demonstrable amounts in Fe agar. Reports in the literature of a frequent occurrence of strains of E. insidiosa which do not form H_2S may be ascribable to the use of a not

sufficiently sensitive medium. *Nielsen* (1966) found that 3 out of 15 strains examined were H_2S negative in the medium employed (iron agar).

Virulence determination on mice proved that 61 strains were strongly virulent, the LD50 being from $0.5 \times 10^{-4.17}$ to $0.5 \times 10^{-8.5}$. One strain (no. 36 which was also rather inactive biochemically) was avirulent, intraperitoneal injection of 0.5 ml of a 24-hr.-old broth culture causing no sign of disease in the mice during the observation period (14 days). This result was rather surprising, since it might have been expected that chronic infections would be caused by weakly virulent organisms.

Virulence determination on pigs was carried out with 7 strains isolated from joints of pigs with arthritis, and with a strongly virulent Swedish strain of E. insidiosa (R/9). As appears from Table 2, 6 of the arthritis strains gave moderate to strong local reactions and/or rise of temperature, while 1 strain was avirulent. The avirulent strain (no. 36) was the same as the one shown to be avirulent for mice.

All strains were examined for production of hyaluronidase (Table 1). Unexpectedly, the highest hyaluronidase content was not found in the strongly virulent strains, but in the strain (no. 36) that was avirulent for mice and pigs.

CONCLUSION

In the present investigations, strains of E. insidiosa isolated from pigs with chronic infection (in this case arthritis) were found not to be different from other strains of this species as regards morphology, cultural features, biochemical reactions and virulence.

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SUMMARY

Sixty-two E. insidiosa strains isolated from joints or regional lymph nodes of pigs were examined from the point of view of morphology, cultural aspects, biochemical activity and virulence. All the strains consisted of gram-positive, short rods, which were similar on solid and fluid media. All strains formed H_2S. Otherwise the biochemical activity was rather low except in 1 strain (no. 18), which was very active. One strain (no. 36) was rather inactive, since it showed no other activity than H_2S formation. This latter strain was the only one that was avirulent for mice. The rest of the strains (61) were strongly virulent for mice (LD50 $0.5 \times 10^{-4.17}$ to $0.5 \times 10^{-8.5}$).

Of 7 strains examined for virulence for pigs by intracutaneous injection of 0.1 ml broth culture, 6 were virulent. The 7th, which was avirulent, was the one that was also avirulent for mice.

SAMMENDRAG

Undersøgelser over Erysipelothrix insidiosa s. rhusiopathiae.
1. Morfologi, kulturelle forhold, biokemi og resistens.

Toogtres rødsygestammer dyrket fra led eller lymfekirtler af grise er undersøgt med hensyn til morfologi, kulturelle forhold, biokemisk aktivitet og virulens.

Alle stammer var gram-positive korte stave, som ikke adskilte sig fra hinanden ved dyrkning på fast og i flydende substrat.

Alle stammer dannede H_2S.

Den biokemiske aktivitet var ret beskeden med undtagelse af én stamme (no. 18), der var meget aktiv.

En stamme (no. 36) var relativt inaktiv, idet den ikke viste biokemisk aktivitet udover at danne H_2S.

Denne stamme var den eneste, der var avirulent for mus. De øvrige 61 stammer var stærkt musepatogene (LD50 $0{,}5 \times 10^{-4{,}17}$ til $0{,}5 \times 10^{-8{,}5}$).

Af 7 stammer, hvis virulens for grise blev undersøgt ved intrakutan injektion af 0,1 ml bouillonkultur, var 6 virulente. Den 7., som var avirulent, var den samme stamme, som var avirulent for mus.

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