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STUDIES ON ERYSIPELOTHRIX INSIDIOSA S. RHUSIOPATHIAE *)

2. SEROLOGICAL STUDY

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For many years the antigenic structure of all known strains of E. insidiosa was considered to be identical. However, *Watts* (1940), in his study of 43 strains of E. insidiosa from America, Japan, England and other parts of Europe, showed that this was not the case, as he was able to divide his strains into 2 groups by means of agglutination tests. Some cross agglutination between the 2 groups was observed, but group specific antisera were obtained by absorption. Specific antisera could also be produced by immunization with boiled antigen.

Atkinson (1941) examined 33 Australian strains of E. insidiosa and produced antisera by immunizing rabbits with boiled antigen and with antigen heated to 56°C for 30 min. There were no qualitative differences between sera produced by the 2 methods. *Gledhill* (1945) examined 31 strains of E. insidiosa, among which there were 6 of *Watts'* strains, 2 strains from the National Collection of Type Cultures, and 3 isolated by *Gledhill* from, respectively, a pig, a duck, and a mouse. Of the remaining 20 strains, 12 were isolated from pigs, 4 from sheep, 2 from mice, 1 from a goose, and 1 from an African jacana.

Using *Watts'* strains, *Gledhill* was unable to confirm *Watts'* finding that group-specific antisera were easily obtainable by immunization with boiled antigen. On the contrary, he found not only that the production of such sera required intensive absorp-

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tion, but also that it was possible to remove all agglutinins by absorption with boiled antigen.

Gledhill demonstrated 2 serological groups in addition to the 2 found by *Watts,* and 24 of his 31 strains could be referred to these 4 groups. Four strains were agglutinated by sera from 2 groups, and 7 strains gave no agglutination with any of the sera.

Dedié (1949) examined 100 strains of E. insidiosa isolated from pigs in Germany and found 2 serological variants: A and B, which were characteristic in containing each a specific acid-soluble antigen. Some strains — N forms — did not possess this antigen.

The antigens were demonstrated by precipitation, agglutination and complement fixation. Of the 100 strains, 55 belonged in type A, 37 in type B, and 8 in type N.

Heuner (1958), studying 147 strains of E. insidiosa of varying origin by precipitation tests on HCl extracts, found that 100 strains belonged in type A and 24 in type B, while 23 gave no precipitation.

Heuner then carried out precipitation tests on CH_3COOH extracts from the same strains and was thereby able to divide them further.

All strains, HCl extracts of which were precipitated by specific A sera were referred to type A_1. Identical results were obtained with CH_3COOH and HCl extracts, although the precipitation reaction was often stronger with CH_3COOH extracts than with HCl extracts.

Type A_2 contained 7 strains that could only by determined as A strains by means of CH_3COOH extracts, no precipitation occurring with HCl extracts. Antisera made with 3 different A_2 strains precipitated only CH_3COOH extracts from A_2 strains. Extracts from A_1 strains were never precipitated by A_2 sera.

The B_1 type included 21 typical B strains, from which precipitinogen was extractable by HCl and CH_3COOH, and 4 strains which could be determined as B strains only by means of CH_3COOH extracts, and which had previously been designated N strains. Sera produced against a single-cell culture of 1 of these 4 strains behaved as typical B_1 sera in the precipitation test.

There were 3 strains which rendered clearly B-precipitinogenic CH_3COOH extracts while HCl extracts of the same strains were precipitated by only a few B sera. Immune sera produced against 2 single-cell cultures of 1 of the strains precipitated

CH_3COOH extracts of typical B_1 strains, but not HCl extracts. This strain and 2 others which behaved in the same way have been placed in a tentative type B_2 on the basis of the agglutination results.

Antisera for a fish strain gave precipitation with HCl and CH_3COOH extracts of the homologous strain only. This strain was regarded as a separate type, designated type C.

Two other fish strains were found, HCl and CH_3COOH extracts of which were precipitated only by the homologous sera. These 2 strains were called D strains.

Nine strains in which no acid-soluble antigen could be demonstrated were designated N strains.

The distribution of *Heuner*'s 147 strains was thus as follows: 100 A_1, 7 A_2, 25 B_1, 3 B_2, 1 C, 2 D, and 9 that contained no acid-soluble antigen, i.e. N. strains.

In the present study, strains of E. insidiosa, isolated from joints or regional lymph nodes of pigs with arthritis, were examined with regard to their antigenic characteristics.

MATERIAL AND METHODS

Sixty-two strains were examined. HCl and CH_3COOH extracts were made from each strain and tested against precipitating antisera for types A_1, A_2, B_1, B_2, C, D, E, and G.

Production of antisera. Four rabbits were immunized with each strain*). The strains were grown in beef broth with 1 % peptone, pH 7.4. After incubation for 18 to 24 hrs. at 37°C, the cultures were centrifuged and the organisms washed twice with physiological saline. The packed, washed organisms were suspended in 0.3 % formol saline to half the original volume. After incubation at 37°C for 18 to 24 hrs. sterility test was made on blood agar plates. At intervals of 4 days, rabbits were injected i. v. with, respectively, 1 ml, 2.5 ml, 5 ml, and 10 ml of the suspension of killed organisms. Blood samples were taken 10 days after the last injection. If precipitating antibodies had formed to such an extent that a clear precipitation line would appear between antigen and homologous serum, the rabbit was killed and its blood collected. If the formation of precipitating anti-

*) Type strains of E. insidiosa were kindly supplied by Professor *G. Wellmann*, Berlin.

bodies was not satisfactory, the above-mentioned course of injections was repeated with living organisms.

Production of precipitating antigen. After 2 mouse passages the strains under study were grown in beef broth with 1 % peptone, pH 7.4. After incubation at 37°C for 24 hrs., the culture was centrifuged and the organisms washed twice with physiological saline.

For production of HCl extract (*Lancefield* 1928) the washed and packed organisms were suspended in 0.05 N-HCl to a volume corresponding to 5 % of the original broth culture. The suspension was boiled for 15 min. on waterbath and then centrifuged. The supernatant was neutralized with 5 % Na_2CO_3 solution with phenol red as indicator and centrifuged, for removal of deposit.

CH_3COOH extract was prepared by the following method (*Heuner* 1958): The washed and packed organisms were suspended in 0.033 N-CH_3COOH to a volume corresponding to 5 % of the original broth culture. The suspension was boiled on waterbath for 45 min. and then centrifuged. The supernatant was neutralized and centrifuged as described above.

RESULTS AND DISCUSSION

The greatest specificity of sera was obtained by immunizing rabbits with killed organisms.

Each antiserum was tested against all the strains used for immunization, and the results are shown in Table 1.

As will be seen, antigen from A_1 organisms precipitated with A_1 serum both in HCl and CH_3COOH extracts, while none of the 2 extracts reacted with A_2 serum. The A_2 type reacted with both A_1 and A_2 sera, but only in CH_3COOH extract.

Antigen from B_1 organisms reacted with B_1 and B_2 sera in CH_3COOH extract, but only with B_1 serum in HCl extract. This was in accordance with *Heuner* (1958). Antigen from B_2 organisms reacted with B_1 and B_2 sera in both HCl and CH_3COOH extracts. This finding deviates from the findings of *Heuner* who got no reaction between the HCl extract and B_2 serum. *Kucsera* (1961) found no differences between *Heuner*'s B_1 and B_2 strains.

The antisera produced against types C, D, E, and G reacted each with its homologous type, but showed no cross reactions. Reaction occurred both in HCl and CH_3COOH extracts.

Table 1. Cross precipitation tests between type strains and their respective antisera.

Antigen	Acid extract	Antisera A_1	A_2	B_1	B_2	C	D	E	G
A_1	CH_3COOH	+	—	—	—	—	—	—	—
	HCl	+	—	—	—	—	—	—	—
A_2	CH_3COOH	+	+	—	—	—	—	—	—
	HCl	—	—	—	—	—	—	—	—
B_1	CH_3COOH	—	—	+	+	—	—	—	—
	HCl	—	—	+	—	—	—	—	—
B_2	CH_3COOH	—	—	+	+	—	—	—	—
	HCl	—	—	+	+	—	—	—	—
C	CH_3COOH	—	—	—	—	+	—	—	—
	HCl	—	—	—	—	+	—	—	—
D	CH_3COOH	—	—	—	—	—	+	—	—
	HCl	—	—	—	—	—	(+)	—	—
E	CH_3COOH	—	—	—	—	—	—	+	—
	HCl	—	—	—	—	—	—	(+)	—
G	CH_3COOH	—	—	—	—	—	—	—	+
	HCl	—	—	—	—	—	—	—	(+)

Table 2. Precipitation tests with type sera on 62 isolates of E. insidiosa.

No. of strains	Acid extract	Antisera A_1	A_2	B_1	B_2	C	D	E	G	Type
1	CH_3COOH	+	—	—	—	—	—	—	—	A_1
	HCl	+	—	—	—	—	—	—	—	
9	CH_3COOH	—	—	+	+	—	—	—	—	B_1
	HCl	—	—	+	—	—	—	—	—	
4	CH_3COOH	—	—	+	+	—	—	—	—	B_2
	HCl	—	—	+	+	—	—	—	—	
32	CH_3COOH	—	—	+	+	—	—	—	—	B_3
	HCl	—	—	—	—	—	—	—	—	
1	CH_3COOH	—	—	—	—	—	—	—	—	B_4
	HCl	—	—	+	+	—	—	—	—	
15	CH_3COOH	—	—	—	—	—	—	—	—	N
	HCl	—	—	—	—	—	—	—	—	

Table 2 shows the results of precipitation tests between acid extracts of the 62 strains and the type-specific sera. As will be seen, 1 strain belonged in type A, subtype A_1. Fifteen strains whose acid extracts did not react with any of the type-specific sera were considered to be without acid-soluble antigen and therefore referred to type N. All the remaining 46 strains were determined as belonging in type B, but could be further divided into subtypes as follows:

In the case of 9 strains, CH_3COOH extracts reacted both with B_1 and B_2 sera, HCl extracts with B_1 serum only. In accordance with *Heuner* these 9 strains are referred to type B_1.

Four strains were characterized by CH_3COOH and HCl extracts giving precipitation with B_1 and B_2 sera. These strains are referred to *Heuner*'s type B_2.

Thirty-two strains, CH_3COOH extracts of which reacted with B_1 and B_2 sera, while HCl extract gave no reaction with any of those 2 sera, are referred to a new subtype, designated B_3.

Finally, there was 1 strain, HCl extract of which reacted with both B_1 and B_2 sera, while CH_3COOH extract did not react with any of the sera. This strain (no. 36, which differs from all the other strains also with respect to virulence and biochemical features (*Nørrung* 1970) is referred to a new subtype, designated B_4.

CONCLUSIONS

Of 62 strains of E. insidiosa originating from pigs with arthritis, 46 could be referred to the serological type B, 15 to type N, and 1 to type A.

The 46 B strains fell into 4 subtypes, 2 of which (B_3 and B_4) have not been described previously. Type B_4 is remarkable in rendering precipitable antigen in HCl extracts, but not in CH_3COOH extracts.

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SUMMARY

Sixty-two strains of E. insidiosa isolated from joints or regional lymph nodes of pigs with arthritis were examined for their antigenic characteristics. HCl and CH_3COOH extracts were made from all strains and precipitation tests performed with these extracts and rabbit antisera for types A_1, A_2, B_1, B_2, C, D, E, and G.

The type distribution of the strains was as follows: 1 belonged in type A, 46 in type B, while 15, which did not contain demonstrable acid-soluble antigen, were designated N strains. The 46 type B strains could be divided into 4 subtypes on the basis of the patterns of reaction between, on the one hand HCl and CH_3COOH extracts, and, on the other hand, B_1 and B_2 sera. Thirty-two strains reacted with both B_1 and B_2 sera in CH_3COOH extracts, but not in HCl extracts; these strains were referred to a new subtype, B_3. Nine strains, which reacted with B_1 serum in HCl and CH_3COOH extracts and with B_2 serum in CH_3COOH extracts only, were referred to subtype B_1. Four strains that reacted with B_1 and B_2 sera in both extracts were referred to subtype B_2. The only representative of the 4th subtype was an interesting strain which in previous studies had been shown to differ from the rest of the strains in other ways, too. This strain reacted with both B_1 and B_2 sera, but only in HCl extract.

SAMMENDRAG

Undersøgelser over Erysipelothrix insidiosa s. rhusiopathiae. 2. Serologiske undersøgelser.

Toogtres rødsygestammer isoleret fra led eller regionale lymfekirtler af grise med ledbetændelse er undersøgt for antigene egenskaber. HCl og CH_3COOH ekstrakter blev fremstillet af alle stammer

og præcipitationsprøver udført med disse ekstrakter og kanin antisera over for typerne A_1, A_2, B_1, B_2, C, D, E og G.

Typebestemmelsen gav følgende resultat: 1 stamme hørte til type A, 46 til type B, medens 15, som ikke indeholdt påviseligt syreopløseligt antigen, blev betegnet N-stammer.

De 46 stammer hørende til B-gruppen kunne deles i 4 undertyper på grund af forskelle i reaktionen mellem på den ene side HCl og CH_3COOH ekstrakter og på den anden side B_1 og B_2 sera. Toogtredive stammer reagerede med både B_1 og B_2 sera i CH_3COOH ekstrakt, men ikke i HCl ekstrakt; disse stammer blev betragtet som hørende til en ny undertype, som blev betegnet B_3. Ni stammer, som reagerede med B_1 serum i HCl og CH_3COOH ekstrakter og med B_2 serum i CH_3COOH ekstrakt alene, blev betegnet som hørende til undertype B_1. Fire stammer, som reagerede med B_1 og B_2 sera i begge ekstrakter, blev betegnet som hørende til undertype B_2. Den eneste repræsentant for den 4. undertype var en interessant stamme, som ved tidligere undersøgelser havde adskilt sig fra de øvrige stammer også i andre henseender. Denne stamme reagerede med både B_1 og B_2 sera, men kun i HCl ekstrakt.

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