Acta vet. scand. 1981, *22*, 46—54.

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IMMUNOLOGICAL CROSS REACTIONS OF ANTIGENS OF ERYSIPELOTHRIX RHUSIOPATHIAE AND HEART TISSUE FROM SWINE

By

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BRATBERG, ANNA MARIE: *Immunological cross reactions of antigens of Erysipelothrix rhusiopathiae and heart tissue from swine.* Acta vet. scand. 1981, *22*, 46—54. — Antisera against 4 strains of E. rhusiopathiae and against heart tissues from swine were raised in rabbits and used in gel double diffusion precipitation tests against 10 strains of E. rhusiopathiae of various origins and against heart tissues. Precipitation lines showing cross reactions were found in tests with one bacterial antiserum and with both tissue antisera tested. This indicates a similarity between the antigenic determinants of heart muscle and valves from swine and certain strains of E. rhusiopathiae. The possible relationship between the results and the virulence of the microorganisms is discussed.

endocarditis; Erysipelothrix rhusiopathiae; swine; immunology.

Within the species E. rhusiopathiae there is great variation between strains in biochemical activities and antigenic determinants as well as in virulent properties. The immunological status of the host will be an important factor in determining the course of an infection. The animal may or may not have been previously exposed to the organism and may already have produced antibodies against it. Many factors concerning the interaction of the host and parasite have not been fully investigated (*Wood & Shuman* 1975). Chronic forms of erysipelas in swine with endocarditis and chronic arthritis are occasionally seen some weeks after an outbreak of acute erysipelas in the herd. The delayed manifestations and subsequent histopathological findings have led to a comparison with streptococcal infections in man in which rheu-

matic diseases affecting the heart valves and joints may follow acute infections. The mechanism of human rheumatic disease is thought to be an immunological reaction to connective tissue (*Hoeprich* 1977). An important finding has been the demonstration that streptococcal antigens cross react with antigens of heart and skeletal muscle, elements of connective tissue, mucopolysaccharides on the heart valves, and other tissue components. Human rheumatic endocarditis is an aseptic condition of the connective tissue of the valves, whereas a pathological examination of a erysipeloid swine heart shows an infective endocarditis in which the vegetations on the valves contain microorganisms (*Jubb & Kennedy 1970, Hoeprich*). In the establishment of infective endocarditis, a pre-existing injury of the heart valves, such as rheumatic endocarditis, has been shown to be a predisposing factor in man (*Svanbom & Strandell* 1978, *Hoeprich*). In animals there is no evidence for pre-existing disease of the valves. It is considered that any stress condition that affects the endothelium of the valves will enhance the ability of microorganisms to adhere, thereby establishing an infection. Stress might result from fever and recurrent bacteraemia or an increased work load (*Jubb & Kennedy*). It seems possible that an immunological reaction in the heart valve, with a mechanism similar to the rheumatic reaction, could be the lesion that will initiate the endocarditis caused by E. rhusiopathiae in swine.

The present investigation was designed to show whether immunological cross reactions of antigens of E. rhusiopathiae and heart tissues from swine could be demonstrated by the use of an agar gel double diffusion precipitation test.

MATERIALS AND METHODS

The strains of E. rhusiopathiae included in the experiment and their origins are listed in Table 1. Antigen homogenates were produced by growing the microorganisms on blood agar plates at 37°C for 72 h. The plates were rinsed with psysiological saline, the cells were washed, centrifuged 3 times and then resuspended to an optical density (OD) of 0.3—0.5. The suspensions of bacterial cells were frozen and disintegrated. The homogenates were used as antigens.

Bacterial antigen extracts (Table 2) were produced by cultivating the microorganisms in nutrient broth containing 10 %

Table 1. Strains of Erysipelothrix rhusiopathiae used for production of antigens.

Designation	Origin
2240	Swine, heart valve, endocarditis.
2553	— „ —
10	— „ —
1999	Swine, skeletal muscle, bacteraemia.
24	Swine, labelled type A_1, unknown pathological changes.
14	Lamb, joint, chronic arthritis.
32	Unknown origin, labelled type N.
R	Unknown origin, maintained in laboratory for several years.
S	— „ —
2	— „ —
20 u	— „ —
24 u	— „ —

horse serum at 37°C for 24 h. The cultures were centrifuged at 2000 r.p.m. for 15 min. The bacterial cells were washed and centrifuged twice using physiological saline, then resuspended in distilled water to 1/10 of the original volume. The suspensions were autoclaved at 120°C for 1 h and used as antigens.

Antigens from heart muscle without endothelium (M) and from atrioventricular valves (K) were prepared from the hearts of swine 5—6 months old and collected immediately after slaughter. Approximately 5 g of tissue were cut, rinsed with physiological saline and finely ground. The tissue was washed and centrifuged twice, resuspended in physiological saline at about 3 times the volume of the tissue, frozen, and disintegrated. The homogenates were used as antigens. All types of antigens were stored at —18°C until use.

Antisera were produced in rabbits by using homogenates of bacterial cells and of tissues prepared as described above. Antigens were mixed with the same amount of Freund's complete adjuvant (Bacto) and injected i.c. and s.c. Injections were repeated once weekly for 6 or 7 weeks using incomplete adjuvant. Serum samples then gave distinctly positive reactions with homologous antigens in agar gel diffusion tests. Sera thus obtained were stored at —18°C until use.

Gel precipitation tests were performed on 10×10 cm glass plates. 20 ml gel consisting of 1 % agarose (A 37, Pharmindustrie, Clichy, France) in phosphate buffered saline was poured on to each plate. Systems of wells 3 mm in diameter were cut, giving a

volume of 10 µl and a centre-to-centre distance of 9 mm. Antisera were placed in the centre wells and antigens in the peripheral wells. The plates were incubated in a moist chamber at 4°C, and were examined after 48 and 72 h.

RESULTS

The results of the precipitation tests are listed in Table 2. The patterns of precipitation lines of the 6 antisera investigated are as follows:

Antiserum R gave 1 or 2 precipitation lines against all the bacterial antigens, both homogenates and extracts, with the exception of No. 14. M and K showed no precipitation lines. Dif-

Table 2. Results of double diffusion gel precipitation tests.

Antigens	Antisera Produced against					
Designation	E. rhusiopathiae strains				tissues	
Homogenates of E. rhusiopathiae	R	S	20 u	24 u	M	K
2240	++	+	++	—	++	+
2553	+	+	++	—	+	—
10	+	+	—	+	—	+
1999	++	+	—	—	—	+
24	+	—	—	—	—	—
14	—	—	—	—	—	—
32	+	—	—	—	—	+
R	++	+	++	+	++	+
S	+	+	++	—	++	+
2	+	+	—	—	—	+
Extracts of E. rhusiopathiae						
Ex 10	+	—	—	—	—	—
Ex 24	++	—	—	—	—	—
Ex 14	+	—	—	—	—	—
Ex 32	+	—	—	—	—	—
Ex 2	++	—	—	—	—	—
Homogenates of tissues						
M	—	—	++	—	++	++
K	—	—	+	—	++	++

++ indicates two precipitation lines,
+ indicates one precipitation line,
— indicates no visible precipitation line.

ferent antigen arrangements revealed that the inner precipitation line was continuous for all positive antigens. The homogenates showing 2 precipitation lines also shared the outer line, while the second line of some of the antigen extracts was placed close to the inner line (Fig. 1).

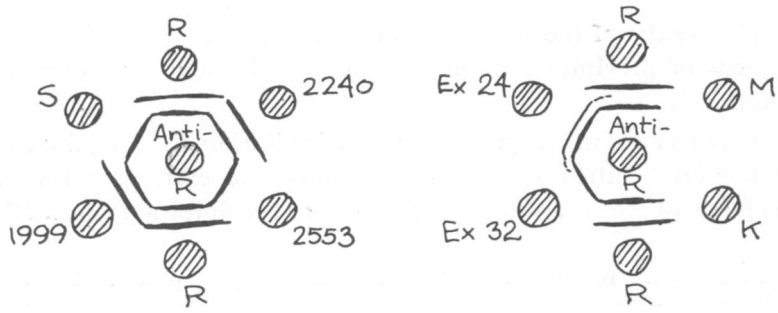

Figure 1. Gel precipitation testing E. rhusiopathiae antiserum R (center wells) against homogenates and antigen extracts of various strains of E. rhusiopathiae (R, 2240, 2553, 1999, S, Ex 32, Ex 24) showing 3 systems of precipitation lines. The antigens from heart tissues (M, K) show no precipitation lines.

Antiserum S showed 1 system of precipitation lines against 7 of the bacterial homogenates. M and K showed no precipitation line (not shown).

Antiserum 20 u showed 2 systems of precipitation lines against 4 of the bacterial homogenates. The tissue antigens M and K shared the inner line, and M also took part in the outer line (Fig. 2).

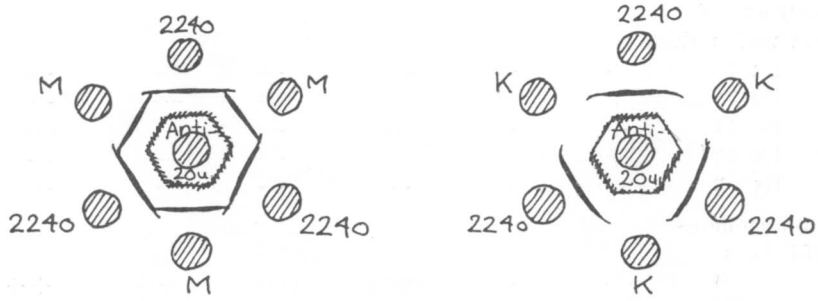

Figure 2. Gel precipitation testing E. rhusiopathiae antiserum 20 u (center wells) against homogenates of a strain of E. rhusiopathiae (2240), heart muscle antigen (M), and heart valve antigen (K) showing 2 precipitation lines for 2240 and M and 1 line for K.

Antiserum 24 u showed 1 weak precipitation line against 2 bacterial homogenates (not shown).

Antiserum M showed precipitation lines against the same 4 antigens as antiserum 20 u, with nearly the same pattern (Fig. 3).

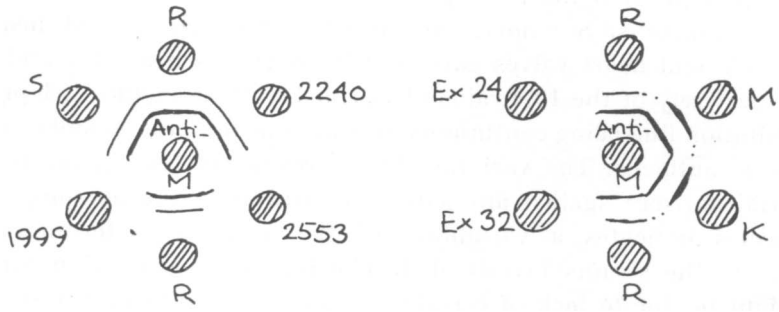

Figure 3. Gel precipitation testing heart muscle antiserum (center wells) against homogenates and antigen extracts of various strains of E. rhusiopathiae (R, 2240, 2553, 1999, S, Ex 32, Ex 24) and against tissue antigens (M, K) showing 2 systems of precipitation lines.

Antiserum K showed 1 precipitation line against 7 of the bacterial homogenates, 6 of them the same as those that were positive with antiserum S. Against the tissue antigens M and K there were 2 lines (Fig. 4).

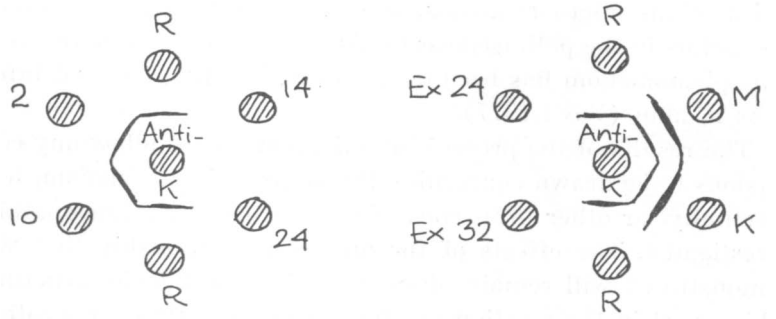

Figure 4. Gel precipitation testing heart valve antiserum (center wells) against homogenates and antigen extracts of various strains of E. rhusiopathiae (R, 14, 24, 10, 2, Ex 32, Ex 24) and against tissue antigens (M, K) showing 2 systems of precipitation lines.

DISCUSSION

The results demonstrate immunological cross reactions between the heart muscle and heart valves from swine and the microorganism E. rhusiopathiae. The reaction was demonstrated in 2 ways: one of the antisera produced against 4 separate strains of E. rhusiopathiae cross-reacted with antigens of heart tissues, the precipitation lines being continuous with lines against bacterial antigens. Secondly, the antisera produced against heart muscle and heart valves gave positive reactions against 4 and 7 respectively of the 10 strains of E. rhusiopathiae included, 1 precipitation line being continuous with lines against the homologous tissue antigens. The variation in the results of testing the bacterial antisera against antigens of 10 strains of the homologous species indicates a variability of the antigenic determinants among the strains investigated. The lack of precipitation lines might be due to lack of certain antigenic determinants for some of the strains, or, the antigenic determinants taking part in the precipitation reactions might have been at too low a concentration to produce a visible reaction. The reactions with antisera produced against tissues showed a similar variation. It is noteworthy that there seems to be a similarity in the pattern of positive reactions for heart muscle antiserum M and the bacterial antiserum 20 u, and for heart valve antiserum K and the bacterial antiserum S. It is possible that mammalian tissue with its huge numbers of potential antigenic determinants may have some determinants in common or similar in structure to those of infectious microorganisms, and that immunological reactions are factors in the pathogenesis of diseases in the connective tissue. This phenomenom has been demonstrated in streptococcal infections in man (*Vosti* 1977).

The results of the present investigation do not allow any conclusions to be drawn concerning the pathogenic mechanism, host specificity, or other properties of the strains of E. rhusiopathiae investigated. The effects of the antigenic relationship that was demonstrated will remain obscure until the antigenic structures taking part in the reaction are further characterized or localized in the tissues. In a preliminary study at our laboratory, sections of normal and inflamed endocardial heart valves were treated with and indirect immunofluorescence stain by the method of *Espinosa & Kaplan* (1971). A localization of a possible positive reaction was not obtained because of unspecific staining of

various structures in the tissues. Further investigations also include correlation to other characteristics of the strains of E. rhusiopathiae. Tests for biochemical and enzymatic activity and virulence might reveal details in the establishment of various forms of the infection. The strains designated 2, 10, 14, 24, and 32 were also included in an in vitro test for the adherence of microorganisms to endocardial surfaces of heart valves from swine. The results showed a significant difference ($P < 0.01$) in adherence for the five strains, strains No. 10 and No. 24 showing the highest number of adhered microorganisms (*Bratberg* 1981).

Another important factor in any investigation would be the serotypes of the strains. Of the E. rhusiopathiae strains included, 2 were typed according to systems used earlier. The standardized system of *Kucsera* (1973) now in use gives a more extensive and precise classification of the genus. Strains isolated from endocarditis in swine have been classified as serotypes 1a, 1b, 2a and 5 (*Kucsera* 1979, *Cross & Claxton* 1979). The antigen extracts used in this study were prepared according to the extraction method for this serotyping test. One of the bacterial antisera showed positive reactions with all extracts investigated.

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SAMMENDRAG

Immunologiske kryssreaksjoner mellom antigener fra Erysipelothrix rhusiopathiae og hjertevev fra gris

Antisera mot 4 Erysipelothrix rhusiopathiae-stammer og mot hjertevev fra gris ble framstilt på kanin og brukt i agargel dobbeldiffusjonsprøver mot 10 E. rhusiopathiae-stammer av forskjellig opprinnelse og mot hjertevev. Kryssreaksjoner ble påvist i prøver med et bakterieantiserum og med begge vevsantisera som ble undersøkt. Dette tyder på likhet i antigene determinanter fra hjertemuskulatur og hjerteklaffer og visse stammer av E. rhusiopathiae. Mulighetene for å korrelere resultatene til forskjellige egenskaper av betydning for rødsykebakterienes virulens er kort diskutert.

(Received October 17, 1980).

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