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SELECTIVE ADHERENCE OF ERYSIPELOTHRIX RHUSIOPATHIAE TO HEART VALVES OF SWINE INVESTIGATED IN AN IN VITRO TEST

By
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BRATBERG, ANNA MARIE: *Selective adherence of Erysipelothrix rhusiopathiae to heart valves of swine investigated in an in vitro test.* Acta vet. scand. 1981, 22, 39—45. — An in vitro test for the adherence of microorganisms to epithelial surfaces was performed in an attempt to illustrate the initial steps in the establishment of infectious endocarditis. Preparations of fresh heart valves of swine were incubated in suspensions of Erysipelothrix rhusiopathiae. There was a significant difference ($P < 0.01$) in the degree of adherence between the 5 strains of E. rhusiopathiae investigated. The highest degree of adherence was found in an isolate from an endocardial vegetation and in a strain labelled type A_1, both originating from swine. Scanning electron microscopy showed the microorganisms distributed on the endocardial surface and in large numbers at the base of the chordae tendinae.

selective adherence; Erysipelothrix rhusiopathiae; infectious endocarditis.

Endocarditis caused by Erysipelothrix rhusiopathiae is one of the clinical manifestations of chronic erysipelas in swine. The inflammation begins at the margin of the valve and develops into large vegetations containing microcolonies of the organisms. Death usually occurs due to the development of valvular insufficiency or the generation of emboli. Theoretically, 2 routes are possible for the implantation of the microorganisms on the endocardium: the interstitial capillary bed beneath the endothelium, and the blood within the endocardial lumen. The fact that the infection typically begins at the free margin, the least vascu-

larised part of the valve, and that even valvular prostheses can form a base for vegetations, makes it probable that the microorganisms enter the endothelium from the endocardial blood stream (*Hoeprich* 1977).

The microorganism occurs in the blood during acute stages of erysipelas. It may occasionally enter the blood via the tonsils and epithelium of the intestine, but without causing clinical disease (*Jubb & Kennedy* 1970). Specific conditions seem to be required for the adherence of the microorganism to the endothelium at this particular site and for it to be retained there, resisting removal by the flowing blood. Selective adherence has been described by several authors for various bacterial infections. *Frost et al.* (1977), studying mastitis provoking bacteria in the bovine mammary gland, found a greater degree of adherence among the most pathogenic strains of microorganisms. They also found a special type of mammary epithelial cell, which the bacteria particularly adhered to. *Jones & Rutter* (1972) found that the adherence of Escherichia coli to the intestinal epithelium in piglets was associated with the K88 antigen on the surface of the bacterial cells. In calves the K99 antigen of E. coli functions in a similar way (*Burrows et al.* 1976). When studying the adherence of Streptococcus pyogenes to epithelial surfaces of the mouth and throat, *Ellen & Gibbons* (1972) showed that the attachment was mediated by the M-protein surrounding the bacterial cell.

Within the species E. rhusiopathiae there is a great variation in biochemical activities and antigenic structures, as well as in virulence (*Nørrung* 1970a, b). Many authors have tried to find specific characteristics related to the various clinical forms of erysipelas. Factors in the host or the environment might also influence the establishment of the infection in various organs (*Wood & Shuman* 1975). Specific factors characterizing the endocarditis provoking strains of E. rhusiopathiae have not been fully investigated and conditions that mediate adherence are not known.

The present investigation is designed to show whether a selective adherence of different strains of E. rhusiopathiae to heart valves of swine can be demonstrated. In the in vitro test used, the degree of adherence is estimated by the number of adherent bacteria after incubation in suspensions of the microorganisms.

MATERIALS AND METHODS

Five strains of E. rhusiopathiae with the following characteristics were investigated:

Strain No. 2 — Unknown origin. Maintained for several years in the laboratory.

„ 10 — Isolated from the heart valve of a pig with endocarditis.

„ 14 — Isolated from the joint of a lamb with chronic arthritis.

„ 24 — Isolated from swine, labelled as type A_1.

„ 32 — Unknown origin, labelled as type N.

The organisms were grown in Todd-Hewitt Broth (Oxid) at 37°C for 18 h. The cultures were centrifuged, washed once in phosphate buffered saline (PBS) and resuspended to approximately 10^5 organisms per ml.

Atrioventricular valves of apparently healthy pigs, 5—6 months old, were collected immediately after slaughter, with 1 sample from each animal in separate tubes. Five parallel tubes were set up for each strain of E. rhusiopathiae. The valves were rinsed in PBS and incubated with the bacterial suspension under slow agitation at 37°C for 30 min. The valves were then washed 6 times with PBS as described by *Frost et al.* (1977). Pieces of approximately 1 cm^2, were cut from the margin of the valves and ground in PBS. Serial dilutions were made of the suspension and 0.1 ml was spread on blood agar plates. The number of colonies was counted after incubation. The results were examined statistically using an analysis of variance, which compared the variance among the strains and the variance for the individual strains.

Selected specimens were fixed in Karnovsky's fixative (*Karnovsky* 1965) at 4°C overnight, dehydrated in acetone and critical point dried using carbon dioxide as the transitional fluid. The dried specimens were attached to metal stubs with silver paste and coated with gold in a vacuum evaporator. The coated samples were examined in a Jeol 50 A scanning electron microscope (SEM). Photographs were taken on Polaroid Type 52 film.

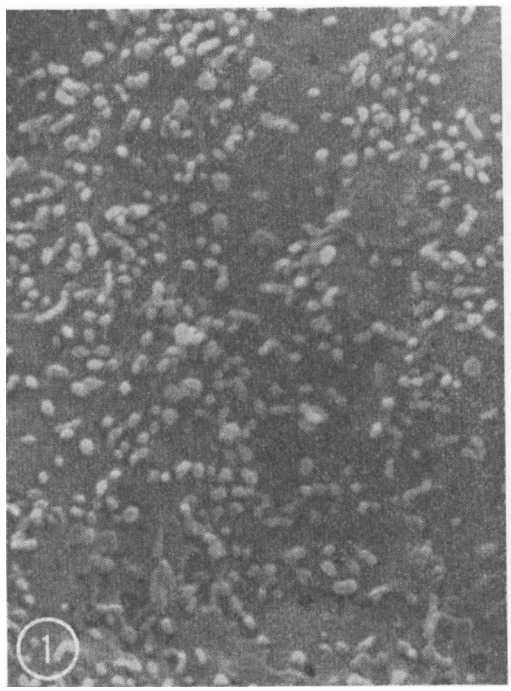
RESULTS

Counts of adhered E. rhusiopathiae cells per cm^2 of heart valve as an average of 5 parallel tests are given in Table 1. Strains

Table 1. Total counts of adhered E. rhusiopathiae cells per cm^2 of heart valve in 5 parallels.

Strain No.	Counts of bacteria per $cm^2 \times 10^2$	
	$\bar{x}$	s
2	366	273
10	1500	890
14	211	123
24	1480	754
32	720	278

No. 10 and No. 24 showed the highest number of adherent bacteria, strain No. 32 an intermediate number and strains No. 2 and No. 14 the lowest numbers. The statistical test showed a significant difference in quantity of adherent microorganisms between the strains of E. rhusiopathiae tested. The variance ratio



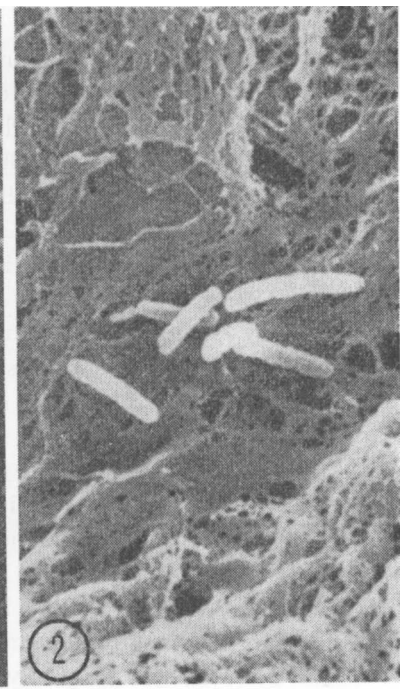


Figure 1. Heart valve, control. Endothelial cells with microvilli form the endocardial surface, SEM × 10,000.

Figure 2. Heart valve, incubated. E. rhusiopathiae adhering to the endothelium. The endothelial cells are damaged showing dicontinuity and loss of surface structures, SEM × 10,000.

was 6.05, $P < 0.01$. Scanning electron microscopy revealed that the surface of the untreated valve was covered with homogenous endothelial cells with small microvilli as shown in Fig. 1. Incubation and rinsing appeared to damage the surface structures as many endothelial cells had started to detach and showed a loss of microvilli. The microorganisms were distributed all over the valve, but were found in their largest numbers at the base of the chordae tendinae. The microorganisms were seen on the endothelium in groups or individually as shown in Fig. 2. Adherence to special cells or cell types could not be demonstrated.

DISCUSSION

The results indicate a significant difference in adherence between the strains of E. rhusiopathiae tested when estimating the degree of adherence as the number of adherent bacteria after incubation. The 2 strains isolated from swine showed the highest degree of adherence to the heart valves of swine. One strain was isolated from an endocardial vegetation, while the other was of an unknown pathological origin. Type A_1 however, is representative of virulent strains usually isolated from septicaemic cases (*Wood & Shuman* 1975).

The lowest adherence was found in the strain isolated from a case of arthritis in a lamb and a strain of unknown origin maintained in the laboratory for several years. This usually brings about a decline in virulent properties. The strain showing intermediate adherence belonged to the N-group, which is characterized by a lack of the antigenic structure normally associated with virulence. Type N, however, has also been isolated from cases of chronic erysipelas (*Nørrung* 1970 b, *Wood & Shuman*). Further correlation of the difference in adherence with other characteristics will require additional investigations of the 5 strains that were tested. An investigation of the antigenic type according to the methods of *Kucsera* (1973) would be of particular interest, as surface antigens of other species of bacteria have been found to bind by selective adherence to epithelial surfaces (*Ellen & Gibbons* 1972, *Jones & Rutter* 1972). A correlation with known structures in the microorganism might provide information as to the type of binding between the endothelial cell and the microorganism. A more marked difference in adherence might be obtained by excluding some uncertain factors from the in vitro test. In this investigation each valve originated

from a different animal. The influence of antibodies to E. rhusiopathiae produced by some animals on adherence of the microorganism is not known. Production of areas of 1 cm^2 for bacterial counts was difficult to standardize because of the irregular surface of the valves.

Frost et al. (1977) found that the microorganisms mainly adhered to a special type of cell in the mammary gland and to detached, damaged cells. In the present investigation the microscopic structures of the endothelium on the valves were too damaged to provide adequate information about the attachment of the bacteria. The thin single cell layer of the endocardium is highly susceptible to damage. The distribution of the microorganisms on the endothelial surface with greatest numbers at the base of the chordae tendinae, might reflect the distribution of endothelial cells normally damaged by wear and tear or for other reasons.

CONCLUSION

The significant difference in the adherence of 5 strains of E. rhusiopathiae to isolated heart valves demonstrated by the in vitro test might indicate, that the selective adherence is a factor in the pathogenesis of endocarditis, where adherence is an initial step in establishing the infection.

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SAMMENDRAG

Selektiv adhesjon av Erysipelothrix rhusiopathiae til hjerteklaffer fra gris belyst i et in vitro-forsøk.

Det er utført et in vitro forsøk for adhesjon av Erysipelothrix rhusiopathiae til overflaten av hjerteklaffer for å illustrere det innledende trinn i utviklingen av infeksiøs endokarditt. Ferskt materiale av hjerteklaffer fra gris ble inkubert i oppslemminger av bakterier. Kimtellinger viste en signifikant forskjell ($P < 0,01$) i adhesjon mellom 5 E. rhusiopathiae-stammer av forskjellig opprinnelse. Størst adhesjon viste en stamme isolert fra en endokardial påleiring og en type A_1-stamme, begge isolert fra gris. I scanning elektron mikroskopi sås mikroorganismene spredt over den endokardiale overflate og i størst antall ved basis av chordae tendinae.

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