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Erysipelothrix rhusiopathiae Infection

Clinical and Gross Pathology, and Bacteriology

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1. Introduction

Erysipelothrix rhusiopathiae is a slender, Gram-positive, non-motile, rod-shaped organism 0.5–2.5 µm by 0.2–0.4 µm, occurring singly in small clusters, and short chains. Occasionally, longer and filamentous forms are observed, and their frequency and length increases with the age of the culture (Jones, 1986; Wood, 1986).

Although growth occurs on ordinary media at room temperature, optimal growth is obtained at 37°C. The organism shows a tendency to be microaerophilic on primary isolation, and indeed prefers such conditions. It is able to grow both aerobically and anaerobically.

E. rhusiopathiae may infect a wide range of mammals, wild and domestic birds, and fish. Depending on the environment, the organism can survive for long periods outside a host. However, there is no evidence to indicate that it can live as a saprophyte. Many pigs are tonsillar carriers of the organism and may pass it in their faeces. The continued presence of the organism in the soil is the result of recontamination by such carriers. In soil *E. rhusiopathiae* dies out at a logarithmic rate which is proportional to the environmental temperature.

2. Clinical Signs

2.1. Pigs

The clinical manifestations of swine erysipelas are usually classified under the following headings (Buddle, 1985; Eamens, 1987).

2.1.1. Peracute septicaemia

Peracute septicaemia is characterised by abrupt onset of severe septicaemia with sudden death, particularly seen in younger animals. Affected pigs frequently exhibit skin discolouration with reddening or purplish discolouration of the ears and underline. Animals surviving the septicaemia often later exhibit signs of acute disease.

2.1.2. Acute septicaemia

Acute septicaemia is characterised by less severe septicaemia, with pyrexia, weight-bearing lameness and reluctance to move. There is often inappetence and depression. Urticaria is frequently seen three to five days post-infection and then disappear. Some affected pigs die of septicaemia, showing skin discolouration (see 2.1.1.).

2.1.3. Subacute septicaemia

Subacute septicaemia is less severe in its manifestations than the acute form, with few skin lesions.

2.1.4. Chronic septicaemia

Chronic septicaemia often follows clinically apparent acute or subacute forms, but may develop from subclinical or inapparent infection. It may be characterised by cutaneous, cardiac or arthritic symptoms, or any combination of these. Signs of lameness due to synovitis or arthritis occur four to five weeks post-infection.

While infection occurs in pigs of all ages, the most susceptible are those from three to 12 months of age, and pregnant sows. Mobility is difficult to assess since clinically inapparant infection is common. In the acute forms it can approach 100%. Mortality is usually low but in rare instances can reach 75%.

2.2. Cattle

A non-suppurative arthritis or polyarthritis is occasionally observed in calves. Clinical erysipelas in adult cattle has not been recorded but the organism has been isolated from the tonsils of healthy cattle and from endocardial lesions at autopsy.

2.3. Sheep

2.3.1. Arthritis/polyarthritis in lambs.

Commonly occurs after marking, less commonly after birth associated with an umbilical infection. Up to 50% of a flock may become affected and although the mortality rate is low, some lambs will lose weight and develop chronic arthritis with swollen joints. Signs appear about 14 days after birth or docking. Arthritis and polyarthritis can also occur in weaners and adults.

2.3.2. Post-dipping laminitis

The use of plunge baths as sheep dips may be followed by a high incidence of laminitis if the insecticide solution does not contain a suitable disinfectant. Infection occurs through skin abrasions and causes a cellulitis with extension to the laminae of the feet but without involving the joints. Up to 90% of a flock may be affected, although the incidence is usually about 25%.

Severe lameness begins two to four days after exposure, usually in one leg, sometimes in all four. Affected legs are hot and slightly swollen from the coronet to halfway up the metatarsus or metacarpus and much body condition is lost. Deaths are rare except in recently weaned lambs which may become septicaemic, exhibiting fever, malaise and anorexia.

2.4. Avian Species

Erysipelas in birds is generally an acute fulminating infection of individuals within a flock. Turkeys are the avian species most commonly affected although occasional economically significant outbreaks in chickens and ducklings have occurred. Outbreaks have also been reported in pheasants.

E. rhusiopathiae is pathogenic for turkeys of any age, causing acute septicaemia; or urticaria; or endocarditis with gradual emaciation, weakness, signs of anaemia and sudden death. It may also cause infertility in male turkeys.

The main clinical signs in chickens are general weakness, depression, diarrhoea and sudden death. Production of laying chickens may be decreased.

Affected ducks, pheasants and quail generally are depressed, have diarrhoea and die suddenly.

Infection is very common in some zoo birds, e.g. tawny frogmouths in Australia. In the United Kingdom it has been isolated from many species of wild birds, including sparrows and pigeons.

2.5. Other Animals

E. rhusiopathiae has been isolated from the liver of a goat and from the bandicoot, rat and brown snake. The presence of the organism in fish slime is probably due to *post mortem* contamination of fish with infected material.

3. Pathology

3.1. Gross Pathology

3.1.1. Pigs

The lesions of septicaemia are not pathognomonic for erysipelas, since similar changes occur in many acute diseases of the pig. The urticaria, however, is pathognomonic. They consist of either rectangular or rhomboidal lesions which are slightly raised, often pale in the centre and surrounded by a narrow zone of congestion, but occasionally with a central purplish–red 'bulls-eye' surrounded by a pale 'halo'.

A cauliflower-like verrucose endocarditis is occasionally encountered in pigs . The lesion is more commonly found on the mitral valve than any other part of the endocardium and consists of foci of young granulation tissue surrounded by masses of fibrin. The commonest causes are *Streptococcus* spp. and *E. rhusiopathiae.*

In acutely affected joints, there is an increased volume of synovial fluid. Initially, the synovial fluid is serofibrinous, but as the inflammatory reaction proceeds, it becomes serosanguinous. In a normal joint, the synovial membrane is smooth and glistening and the villi are not discernible to the naked eye. In *E. rhusiopathiae* synovitis, the villi proliferate and increase in size until some become finger-like and up to 2 cm long. In more progressive cases, at the synovial membrane–cartilage junction, the synovial membrane forms a vascular fringe which advances over the cartilage and invades it to a variable depth. The lymph nodes draining affected joints are a bluish–violet colour, enlarged, oedematous and congested.

The acute synovitis caused by *E. rhusiopathiae* is similar to that caused by: (a) *Pasteurella multocida;* (b) *Haemophilus suis;* (c) *Mycoplasma hyorhinus;* or (d) *Mycoplasma hyosynoviae.* However, in the chronic stages, *Erysipelothrix* arthritis is associated with much more periarticular fibrosis, the synovial membrane is more hyperaemic, the synovial fluid is more serosanguinous, the degree of hyperplasia and hypertrophy of the synovial villi is greater, and the vascular synovial fringe is present .

3.1.2. Cattle

The joint lesions in calves are similar to those in pigs. There is a non-suppurative arthritis with ulceration of the articular cartilages. Polyarthritis is accompanied by lameness, recumbency, fluctuating joint capsules and severe loss of condition. These signs are similar to those seen in arthritis caused by *Mycoplasma* Leach Group 7. Endocardial lesions are similar to those seen in pigs.

3.1.3. Sheep

The joint lesions in lambs are similar to those in pigs — a non-suppurative arthritis or polyarthritis. There is an increased volume of turbid synovial fluid. Thickening of the joint capsule and erosion of the articular cartilages occur.

In sheep suffering from *Erysipelothrix* laminitis, subcutaneous oedema of the affected lower limb is seen, sometimes accompanied by haemorrhage. Inflammation usually extends into the laminae of the feet.

N.B. There is no underrunning of the hoof as in footrot, no abscessation as in foot abscess and no proliferative dermatitis as in strawberry footrot.

3.1.4. Avian Species

The gross lesions in birds are septicaemia with generalised congestion; degeneration of the fat on the anterior edge of the thigh; degeneration and haemorrhage in the pericardial fat; myocardial haemorrhages; increased friability and enlargement of the liver, spleen and kidney.

The following may also be found: fibrinopurulent exudate in joints and pericardial sac; fibrin plaques on heart muscle; thickening of proventriculus and gizzard wall with ulceration; small yellow nodules in the caeca; vegetative endocarditis; dark crusting skin lesions.

In ducks an additional observation has been dark congested areas in the webs of the feet. In turkey toms the snood is often turgid with an irregular reddish–purple colour.

Post insemination losses in turkey hens with peritonitis, subcutaneous perineal haemorrhage or congestion have been reported.

3.2. Histopathology

In general the histopathological findings are not specific for *Erysipelothrix* infection. The synovial lesions in pigs consist of hypertrophy and hyperplasia of synovial lining cells and subsynovial blood vessels. Perivascular accumulations of lymphocytes and plasma cells are present in the subsynovial areas, and these may resemble the germinal centres or lymph nodes. In the chronic stages some arteries and veins become necrotic and are surrounded by palisades of

fibrocytes. Histopathology of skin lesions reveals an area of thrombosis and infarction of the small vessels of the dermis (particularly the superficial dermis).

4. Bacteriology

A rapid presumptive diagnosis can be made by examining Gram stained smears taken from liver, spleen, heart blood or bone marrow for the presence of typical organisms.

E. rhusiopathiae may be isolated from blood and most organs in the septicaemic forms; from skin lesions, and regional lymph nodes in the urticarial form; from valvular lesions in the cardiac form; and from synovial fluid, synovial membrane and regional lymph nodes in the arthritic form. Carriers may occur and in order to isolate *E. rhusiopathiae* from such individuals, multiple samples from various tissues, including tonsils, may have to be cultured. The isolation rate is higher from birds dying of the disease than it is from sick, sacrificed ones.

In turkeys and other avian species it is important to differentiate *E. rhusiopathiae* infection from fowl cholera.

E. rhusiopathiae may be difficult to isolate on primary culture, and special media may have to be used. Broth media based on horse meat serum (HMS) (see 6.1.) give excellent growth from infected tissue.

Suggested selective media are: Broth, Kanamycin–Neomycin–Vancomycin (KNV) (Wood, 1965) and crystal violet–sodium azide (CVA); and agar, CVA agar.

Formulae for these media are given in 6.1.

Synovial fluid or small pieces of synovial membrane may be inoculated directly onto or into media. Larger pieces of synovial membrane and portions of organs such as kidney, liver, lymph nodes and tonsils must be homogenised aseptically before inoculation. From affected joints, homogenised synovial membrane is the material of choice for primary isolation. Synovial fluid can be cultured directly onto blood agar, but the isolation rate is low compared to homogenised synovial membrane subcultured through the special broth media (see 6.1.). Selective media are used if there is any chance that contamination has occurred. When infected homogenised tissue is inoculated into broth enrichment media, growth is usually obtained overnight, but occasionally may not be evident until 3–10 days incubation.

If contaminants grow in either CVA or KNV broths within 18 hours, the broths may be subcultured into KNV broth and onto blood agar, or the original homogenate recultured. KNV broth is preferred, since alpha haemolytic streptococci may grow in CVA media.

The best method for isolation of *E. rhusiopathiae* from synovial membrane or other homogenised tissues is shown in Fig. 1.

The isolate may then be subjected to biochemical tests. The production of acid from glucose, fructose, lactose and galactose, together with negative methyl red, acetylmethylcarbinol and catalase tests, and production of hydrogen sulfide (H_2S), would suggest that the isolate is *E. rhusiopathiae*.

There were 22 recognised serotypes of *E. rhusiopathiae*, based on gel diffusion precipitin tests (Ouchterlony, 1949) using autoclaved aqueous extracts and antisera raised in rabbits (Cross and Claxton, 1979). In pigs in Australia, clinical disease is associated with infections by serotypes 1 and 2. Norrung *et al.* (1987) identified a new serotype 23 isolated from pig/cattle slurry. However, Chinese workers (Xu *et al.*, 1984, 1986) had identified two new serotypes, 23 and 24, and so V. Norrung (pers. comm. 1990) now refers to the Norrung *et al.* (1987) isolate as serotype 25. Japanese reports (Takahashi *et al.*, 1987) had not caught up with this confusion and referred to the slurry strain of Norrung *et al.* (1987) as serotype 23.

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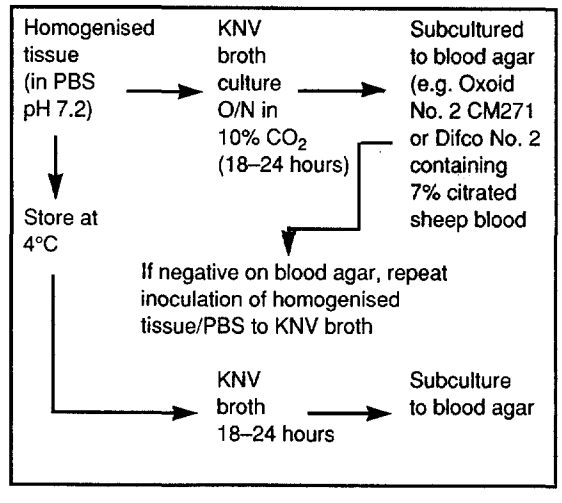

Figure 1. Flow chart for isolation of *Erysipelothrix rhusiopathiae* from infected tissue (PBS, phosphate buffered saline pH 7.2; O/N, overnight).

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6. Appendix

6.1. Media for the Isolation of Erysipelothrix rhusiopathiae

6.1.1. Horse Meat Serum Broth

Fresh horse meat is freed from all fat and debris, finely minced and placed in a stainless steel or glass vessel with two litres of tap water for each kilogram of mince. The mixture is stirred well and allowed to soak at 4°C overnight. The following morning any fat is skimmed from the top, and the mixture stirred and any further floating fat particles removed. The vessel is then placed in a steamer and heated to 95°C for 4.5 hours. The broth is allowed to cool to 70°C and the fluid siphoned off and filtered through a coarse filter paper (e.g. Schleicher and Schuell Grade 613, creped, fast, qualitative). The broth is adjusted to pH 8.0 by the addition of 10 mol/L sodium hydroxide (NaOH). The broth is left overnight at 4°C. Next morning warm the broth in a steamer to 50°C and add Difco proteose peptone, or proteose peptone No. 3, 20 g/L, by lightly shaking over the surface of the infusion while at 50°C. During the addition of peptone to the solution, the following mixture is added to each litre of broth.

Glucose, $C_6H_{12}O_6$	2 g
Sodium bicarbonate, $NaHCO_3$	2 g
NaCl	2 g
Disodium hydrogen phosphate, $Na_2HPO_4.12H_2O$	1 g
(or Na_2HPO_4	0.4 g)

The reaction is readjusted to a pH of 0.8 and is refiltered through coarse filter paper. Equine serum is added to 10% v/v and the mixture filtered firstly through depth filter pads (AMF Cuno 30S), then through either Millipore filters AP 25 > AP 20 > AW 19 > AW 03, or Sartorius GF prefilter cartridge (0.2 µm nominal rating) and then through a 0.22 µm non-sterile cellulose acetate membrane disc or cartridge. It is then filtered through a 0.22 µm sterile membrane filter.

The filtered broth is distributed into sterile bottles of 300–500 mL capacity, and stored at 4°C.

6.1.2 . Selective Media

6.1.2.1. Crystal violet — sodium azide broth

Add 1 mL of 10% sodium azide (NaN_3) (0.15 mol/L) and 1 mL of 0.1% crystal violet to 98 mL of HMS broth. When stored at 4°C this broth lasts four weeks.

6.1.2.2. Crystal violet — sodium azide blood agar

Autoclave 100 mL of Oxoid No. 2 CM271 or Difco No. 2 blood agar base. Allow to cool to 52°C and to 88 mL of blood agar base add 1 mL 10% (0.15 mol/L) sodium azide and 1 mL 0.1% crystal violet. Then add 10 mL sterile citrated sheep or pig blood, mix thoroughly and pour plates.

6.1.2.3. Kanamycin–Neomycin–Vancomycin broth

Make up a solution of HMS broth containing:

Kanamycin	80 mg/100 mL
Neomycin	10 mg/100 mL
Vancomycin	5 mg/100 mL

6.1.2.4. Suppliers

Eli Lilly Australia. Wharf Road, West Ryde, NSW 2114. Tel. (02) 325 4570; Fax (02) 325 4420. Vancomycin hydrochloride.

Sigma-Aldrich. Unit 2/10 Anella Avenue, Castle Hill, NSW 2154. Tel. (008) 800 097; Fax (008) 800 096. Kanamycin, Neomycin.