A MICROBIOLOGICAL STUDY OF POLYARTHRITIS IN SLAUGHTER PIGS

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ABSTRACT: Turner, G.V.S. **A Microbiological study of polyarthritis in slaughter pigs.** *Journal of South African Veterinary Association* (1982) **53** No. 2, 99-101 (En) Department of Veterinary Public Health, College of Veterinary Medicine, Texas A&M University, College Station, Texas 77843, USA.

Arthritic joints obtained from 50 freshly slaughtered pig carcasses condemned for polyarthritis were studied microbiologically. A routine technique was developed for aseptically opening joints to obtain material for microbiological examination. A standard series of culture media for the primary isolation of arthritogenic agents were used in the examination of each affected joint. The microbiological study catered for isolation of the following microorganisms: *Erysipelothrix rhusiopathiae, Corynebacterium pyogenes, Staphylococcus aureus, Escherichia coli, Streptococcus* spp., *Haemophilus* spp., *Mycoplasma* spp., *Salmonella* spp., *Chlamydia,* and viruses.*E. rhusiopathiae* was found to be responsible for 48 % of the cases of arthritis and *Streptococcus* spp., *C. pyogenes* and *S. aureus* for 20 %, 4 %, and 2 % respectively; no microorganisms were cultured from 26 % of the arthritic joints.

INTRODUCTION

Polyarthritis in slaughter pigs is a universal problem. In other countries the occurrence, importance, and aetiology of porcine arthritis is well documented[17]. It is generally accepted that the following microorganisms are the main arthritogenic agents in swine: *Erysipelothrix rhusiopathiae, Corynebacterium pyogenes, Staphylococcus aureus, Haemophilus* spp., *Escherichia coli, Steptococcus* spp., Salmonella spp., and *Mycoplasma* spp.[16]. In many countries *E. rhusiopathiae* is regarded as the major aetiological agent responsible for polyarthritis in slaughter pigs and *Streptococcus* spp. are regarded as the second most common cause[17].

With swine erysipelas being a notifiable disease and a zoonosis and *E. rhusiopathiae* being the main arthritogenic agent encountered in slaughter pigs, it is difficult to understand why the high incidence of arthritis in pig carcasses and the economic implications thereof did not prompt an earlier investigation into the actual aetiology of the problem in South Africa. It therefore became apparent that it was necessary to ascertain the aetiology of arthritis in slaughter pigs encountered at the abattoirs. From the microbiological standpoint it was deemed necessary to develop an efficient and practical method for aseptically opening joints and obtaining microbiological samples for further testing.

MATERIALS AND METHODS

Fore- and hindlegs with unopened intact joints were obtained from freshly slaughtered pig carcasses which had been condemned for polyarthritis. In order to achieve satisfactory results, the fresh material was processed in the laboratory as soon after slaughter as possible. The microbiological examination procedure catered for isolation of the following microorganisms: *E. rhusiopathiae, Streptococcus* spp., *C. pyogenes, S. aureus E. coli, Haemophilus* app., *Mycoplasma* spp., *Salmonella* spp., *Chlamydia*, and viruses. No literature reference to *Chlamydia* or viruses as arthritogenic agents in swine was found. Nevertheless, because of the ubiquitous nature of the *Chlamydia* organisms and viruses, an isolation procedure for these types of microorganisms was included.

A standard series of culture media for primary isolation was used in the examination of each affected joint. The same procedure was carried out step-by-step on each aseptically opened joint. The following culture media were used: blood tryptose agar (BTA) for the isolation of *E. rhusiopathiae, C. pyogenes,* Streptococcus spp., *S. aureus, E. coli,* and *Salmonella* spp.; serum broth for the primary isolation of *E. rhusiopathiae, C. pyogenes, Streptococcus* spp., *S. aureus, E. coli,* and *Salmonella* spp.; chocolate agar for the isolation of strains of *Haemophilus* spp. requiring Factor X for growth; *Staphylococcus aureus* "feeder" strain, known to produce the V factor to ensure growth of *Haemophilus* spp. requiring the V factor; Hayflick's broth, a specific selective medium for the primary isolation of *Mycoplasma* organisms of porcine origin; Chalquest agar, a selective medium used for the primary isolation of *Mycoplasma* organisms, and for subcultures from Hayflick's broth; 7 to 8 day embryonated hen's eggs were used for the recovery of *Chlamydia* organisms from affected joint material[2 3 10]. Stice (PK15) porcine kidney cells were used to screen synovial membrane samples from affected joints for the presence of viruses.

Apparently normal joints were examined in order to adopt a standard routine method of opening the joints, to establish a practical aseptic technique, and to act as controls for the various isolation techniques performed. Ten joints, 2 from each of 5 control pigs, were opened and examined as described below.

In each case the porcine limb or part thereof was fixed in a carpenter's vice. The skin over the affected joint was heat sterilized by thoroughly scorching the area with an iron spatula which had been heated over a Bunsen burner. Using sterile rat-tooth forceps and scalpel, a section of the scorched skin overlying the joint to be opened was removed. The underlying tissues were removed aseptically, and the distended joint capsule exposed. A small incision was made through the distended joint capsule and an aseptic sample of the synovial fluid was taken with a sterile bacteriological swab. Three swabs of synovial fluid were routinely taken per affected joint. The 3 bacteriological swabs were routinely processed as follows: (i) one swab was placed in serum broth. (ii) The second swab was placed in Hayflick's broth. (iii) The third swab was streaked over specific media in the following order: two BTA plates, chocolate agar, and finally, a Chalquest agar plate. The one BTA plate was inoculated by means of a few closely placed parallel strokes of the swab. Using a sterile platinum loop the *Staphylococcus aureus* "feeder" was then streaked at right angles to this[6].

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The joint capsule was further incised and a small piece of capsule was aseptically transferred to serum broth and another placed in Hayflick's broth. A slightly large piece was then removed and firmly streaked sequentially across BTA, chocolate agar, and Chalquest agar. The plating on the Chalquest agar was always performed last because the medium contains pencillin, and it was undesirable for the antibiotic to contaminate antibiotic-free BTA and chocolate agar. For *Chlamydia* and virus detection a piece of joint capsule was ground finely with a sterile pestle and mortar. The ground material was inoculated into the yolksac of 7-8 day embryonated eggs for the isolation of *Chlamydia*, and roller tube cultures of Stice (PK 15) porcine kidney cells were infected as described by Turner[17].

The inoculated solid media were placed in a sealed candle jar in order to create an atmosphere of ± 10 % carbon dioxide. All the media were incubated at 37 °C. Indentification of any growth on BTA was based on the morphological and haemolytic characteristics of the colonies, the production of catalase, the microscopic appearance of a Gram stained smear, and specific biochemical tests based on those recommended in Bergey's Manual of Determinative Bacteriology[1]. The serum broth was examined for signs of growth 24 hours after inoculation. If there was no growth, the serum broth was incubated for an additional 24 hours before subculturing onto BTA. The BTA plate was then examined as described above.

The Chalquest agar was examined under a dissecting microscope for typical *Mycoplasma* colonies. On the second day after inoculating the Hayflick's broth, a subculture was made from the broth onto Chalquest agar. The agar was then examined 7 days later as described above. The Hayflick's broth was discarded at this stage. Where no growth was noted on the Chalquest agar after 7 days the medium was incubated for an additional 7 days.

RESULTS

None of the ten apparently normal joints used as controls yielded any microbiological growth. The results of the microbiological investigation are summarized in Table 1. *E. rhusiopathiae* was found to be responsible for 48 % of the cases of arthritis and *Streptoccus* spp., *C. pyogenes,* and *S. aureus* for 20 %, 4 % and 2 % respectively; no microorganisms could be cultured from 26 % of the arthritic joints. The microorganisms were isolated from both the synovial fluid and synovial membrane samples which had been inoculated onto BTA and into serum broth.

No isolation of *Haemophilus* spp., *Mycoplasma* spp., and *Chlamydia* organisms was made. No evidence of cytopathic changes was noted in the Stice porcine kidney cell cultures and the material was therefore considered free of virus.

DISCUSSION

Certain *Mycoplasma* spp. are known arthritogenic agents in swine and can bring about joint lesions which are difficult to differentiate from those caused by other arthritogenic agents [9 14]. For this reason it was considered necessary to screen the affected joints in this survey for the possible presence of *Mycoplasma* organisms as the causative agent. The complex growth requirements of *Mycoplasma* organisms necessitated the use both liquid and solid media. Hayflick's broth is a suitable enrichment medium for most *Mycoplama* organisms. Chalquest agar is also a suitable medium, especially for the subculture from PPLO broth and facilitates the microscopic detection of the characteristic small colonies[10].

The fact that no *Mycoplasma* were isolated from these media makes it unlikely that *Mycoplasma* were present in the arthritic lesions. *Haemophilus* spp. are known arthritogenic agents which can cause lesions very similar to that caused by other arthritogenic agents[9]. For this reason 2 selective media were utilized for the isolation of *Haemophilus* spp. The fact that no *Haemophilus* spp. could be isolated from the joint material is therefore considered of some signifcance. *Chlamydia* organisms are known to cause polyarthritis in lambs and calves and have been isolated from affected joints in these animals[9 14]. To establish whether *Chlamydia* were causing the arthritis in this study, cultural methods capable of supporting growth of these organisms were included. The negative findings are of significance.

To facilitate the isolation of organisms it is generally recommended that both synovial fluid and a portion of the affected synovial membrane be used as inocula[18]. Because of the scarcity of organisms in some of the more chronic forms of arthritis, it is also recommended that an enrichment medium such as serum be used for primary isolation[15].

It is interesting to note that in this study there was no significant difference between the results obtained from the use of either serum broth or BTA for the primary isolation of *E. rhusiopathiae, Steptococcus* spp., *C. pyogenes,* and *S. aureus.* Similary, no significant difference resulted from the use of either synovial fluid or synovial membrane as inoculum.

Various techniques have been employed for the isolation of *E. rhusiopathiae.* Some workers advocate the use of enrichment media to enhance the possiblity of isolating *E rhusiopathiae* from affected material[15 18]. The use of selective media containing substances such as sodium azide and crystal violet are widely advocated, especially in order to eliminate much of the difficulty in culturing *E. rhusiopathiae* from contaminanted material[11 18]. The fluorescent antibody technique is considered less accurate that cultural methods for the detection of *E. rhusiopathiae* and is not regarded as a satisfactory test for routine use in the diagnostic laboratory[8]. In spite of the fact that in some cases the number of colonies on primary plates may be rather low, the use of blood agar is regarded as a useful medium for the primary isolation of *E. rhusiopathiae* organisms[11]. Because of the reliability of the aseptic technique adopted in this study, it was thought unnecessary to use an additional selective medium merely for the specific isolation of *E. rhusiopathiae.* The use of BTA and serum broth was adequate for the isolation of *E. rhusiopathiae,* as well as catering for organisms such as *C. pyogenes, Streptococcus* spp., *S. aureus,* and *E. coli.*

In this study *E. rhusiopathiae* was found to be the most common isolate. Some workers have, however, also observed that a substantial number of joints showing arthritis failed to yield organisms on culture[5 12 13]. Connell et al. found that a significant percentage of pathological joints did not yield *E. rhusiopathiae* even though the arthristis was initiated by this organism[4].

There are various theories on why the causative organisms cannot be isolated from a numbe of cases of chronic porcine arthritis. It has been postulated that although primarily due to *E. rhusiopathiae,* the arthristis is perpetuated by immunological phenomena[7]. The failure to isolate organisms has also been attributed to the lack of sensitivity of the bacteriological techniques employed[15 18]. With chronicity, bacteria are commonly more difficult to recover from arthritic joints and there may be an element of chance attached to the presence or absence of viable organisms at the site where the bacterial specimen is taken[15]. Based on the above criteria it may be assumed that *E. rhusiopathiae* was responsible for at least some of the arthritic processes in the 26 % culturally negative joints ecountered in this survey.

Based on the fact that the joints of all 10 control pigs were found to be sterile and the absence of obvious contaminants during the entire course of the study, the routine technique used for aseptically opening joints must be regarded as being efficient and practical. For primary isolation of the arhritogenic agents listed, the standard series of culture media employed proved to be the most practical for this study.

Table 1: RESULTS OF MICROBIOLOGICAL EXAMINATION OF ARTHRITIC PORCINE JOINTS

Isolations	Number Carcasses Affected	%
Erysipelothrix rhusiopathiae	24	48
Streptococcus spp.	10	20
Corynebacterium pyogenes	2	4
Staphylococcus aureus	1	2
Negative	13	26
Total Number of Carcases Examined	50	100

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ABSTRACT: Boomker, J., Horak, I.G. & de Vos, V., 1981. **Paracooperiodes peleae gen. et sp. n. (Nematoda: Trichostrongylidae) from the vaal ribbok, *Pelea capreolus*** (Foster, 1790). *Onderstepoort Journal of Veterinary Research*, 48, 169-174 (1981).

A new genus and species of trichostrongylid nematode, *Paracooperioides peleae,* was collected from the small intestines of vaal ribbok, *Pelea capreolus* (Forster, 1790), from the Bontebok National Park, Swellendam, Cape Province.

These nematodes are small and slender with a small cephalic inflation. The cuticle bears numerous transverse striations which are more pronouced anteriorly. The dorsal ray is long and is similar to that of *Gazellostrongylus* Yeh, 1956, and *Cooperioides hepaticae* Ortlepp, 1938, but differs in that it bifurcates in its distal quarter. Each branch divides again, giving rise to a thinner, outer branch and a thicker inner branch. The latter recurves upon itself to form a small, elongated knob. The spicules of *Paracooperioides peleae* resemble those of *C. hepaticae* but can be differentiated from them in that they bear small lateral barbs on their tips. Ten longitudinal ridges, supported by sclerotized rods, are present at the middle of the body. In transverse section, *Paracooperioides peleae* is intermediate between *Cooperioides* Daubney, 1933 and *Paracooperia* Travassos, 1935.

ABSTRACT: De Vos, A.J., Bessenger, R. & Banting, L.F., 1981. ***Theileria? taurotragi:* a probable agent of bovine cerebral theileriosis.** *Onderstepoort Journal of Veterinary Research*, 48, 177-178 (1981).

A case of bovine cerebral theileriosis was confirmed at autopsy on a farm where 4 animals out of 70 died. All were less than 2 years old and all showed nervous signs.

Serologically, no evidence was found of *Theileria mutans* or the *Theileria parva* group in young animals born on the farm. Six out of 13 calves 6-9 months of age were, however, serologically positive for *Theileria? taurotragi* and it was concluded this species was the probable cause of death of the 4 animals.