Acta vet. scand. 1983, *24*, 74—83.

From the Department of Food Hygiene, Faculty of Veterinary Medicine, Swedish University of Agricultural Sciences, Uppsala, and Animal Health Service of the Swedish Farmer's Meat Marketing Association, Visby, Sweden.

ISOLATION AND PARTIAL CHARACTERISATION OF BACTERIA RECOVERED FROM ABSCESSES OF NORMALLY SLAUGHTERED PIGS*

By

Anders Engvall and *Olof Schwan*

ENGVALL, A. and O. SCHWAN: *Isolation and partial characterization of bacteria recovered from abscesses of normally slaughtered pigs.* Acta vet. scand. 1983, *24*, 74—83. — One hundred and one pig abscesses of different localisation were examined. Bacteria were recovered from all abscesses. In 68 abscesses a mixed aerobic and anaerobic flora was found. In 22 abscesses solely aerobes and in 11 solely anaerobes were found. Corynebacterium pyogenes and Fusobacterium necrophorum were found in 61 and 45 abscesses, respectively. Hemagglutination titres of C. pyogenes strains were higher with pig erythrocytes than with sheep erythrocytes. Three of 4 isolates of Staphylococcus aureus produced enterotoxin type A.

pig abscess; Corynebacterium pyogenes; Fusobacterium necrophorum; hemagglutination.

Multifactorial infections from which several aerobic and/or anaerobic bacterial species can be isolated have in recent years gained a great deal of interest both in veterinary and human microbiology (*Balows et al.* 1974, *Sørensen* 1978a, *Schwan* 1979a, *Schwan* 1981). Though many reports deal with the aerobic bacterial flora of abscesses in pigs (*De Bruin & Jaartsveld* 1962, *Armstrong & Payne* 1969, *McCracken & McCaughey* 1973, *Hara et al.* 1976, *d'Aubert* 1981), little information is available regarding the anaerobic and mixed bacterial flora in such infections.

* Part of these results were presented at the 14th Nordic Veterinary Congress, Copenhagen, July 1982.

This study was supported by a grant from the Elsa and Ivar Sandberg foundation.

Jones (1980) found a diverse aerobic and anaerobic flora in the majority of abscesses examined and a sporulating Clostridium was the most frequent anaerobe isolated. Recently *Benno et al.* (1982) reported that Bacteroides ruminicola/oralis was the anaerobic species most frequently isolated from abscesses of different localisation in slaughtered pigs.

This paper describes the isolation and partial characterisation of bacteria recovered from 101 abscesses of normally slaughtered pigs.

MATERIAL AND METHODS

Pus material from 101 abscesses was examined. Seventy-three of these were obtained from a local abattoir. The abscesses were excised from affected sites, placed in plastic bags and transported to the laboratory. The surface was flamed and carefully incised using aseptic techniques. Pus was sampled aseptically with a platina loop and subcultured. In addition, material from 28 abscesses was obtained from a distant abattoir. Using aseptic techniques the abscesses were opened and pus was collected with a sterile cotton swab which was placed in a tube with anaerobic transport medium (Port-a-cul, Becton Dickinson AB, Stockholm, Sweden) and mailed to the laboratory. Most mailed samples arrived at the laboratory within 24 h. All were subcultured immediately on arrival.

All samples were inoculated on 2 horse-blood agar plates (5 % (v/v) horse blood) supplemented with haemin, (United States Biochemical Corporation, Cleveland, Ohio, USA), vitamin K_1, (Sigma Chemical Company, St. Louis, Mo, USA), and L-cystein-hydrochloride (Sigma Chemical Company, St. Louis, Mo, USA) mainly according to *Holdeman et al.* (1977). Plates were incubated anaerobically (BAPan) in an anaerobic chamber (Anaerobic system model 1024, Forma Scientific, Marietta, Ohio, USA) and aerobically (BAPae) at 37°C for 72 h. All BAPan were kept in the anaerobic chamber for at least 24 h before inoculated.

All specimen were also inoculated into chopped meat carbohydrates (CMC) broths (*Holdeman et al.*) using anaerobic techniques as described by *Holdeman et al.*, incubated in 37°C for 72 h, and subcultured to BAPan. These were incubated anaerobically at 37°C for 72 h.

Aerobic isolates were identified according to *Cowan* (1977). Streptococcal isolates were serogrouped according to *Lancefield*

(1933). Staphylococcal isolates were tested for enterotoxin production by the method of *Zehren & Zehren* (1968). C. pyogenes and "Corynebacterium species" isolates were tested for hemagglutinating ability of sheep and pig erythrocytes mainly according to *Lindal & Smyth* (1982). Strains were grown in heart infusion broth (Difco) with 10 % (v/v) heat inactivated horse serum (60°C; 30 min), without agitation for 24 h at 37°C. Absorbance of washed suspensions of bacteria was calculated using a spectrophotometer (Digispec, Helena Lab., Texas, USA) and suspensions were adjusted to give uniform densities of bacteria prior to titrations.

All anaerobic isolates were tested for ability to grow aerobically on BAPae and, if negative, subcultured to CMC or pepton yeast glucose (PYG) broths (*Holdeman et al.*), which were incubated until good growth occurred. Production of volatile and nonvolatile short-chain fatty acids and alcohols was analysed with gas liquid chromatography according to *Holdeman et al.* A Pye Unicam (Svenska AB Philips, Stockholm, Sweden) gas chromatograph with flame ionisation detector and equipped with a 2 m$\times$¼″ glass column containing 5 % FFAP on 80/100 mesh Chromosorb WHP was used and run at 110°C.

Biochemical reactions were determined with API 20A (AB Biodisk, Solna, Sweden) and Minitek anaerob II (Becton Dickinson AB, Stockholm, Sweden) minikits, and with PRAS-media (*Holdeman et al.*).

RESULTS

Of 101 abscesses examined, 41 were localized superficially at various sites, 35 involved the pelvic region, 3 involved bone tissue, and 8 and 2 involved the thoracic pleura and knee-joint, respectively and for 12 abscesses localisation was not recorded.

Bacteria were recovered from all abscesses examined. In 68 abscesses a mixed aerobic and anaerobic flora was found. Growth of solely aerobes occurred in 22 abscesses and of solely anaerobes in 11 abscesses.

Solely anaerobes were isolated twice from superficial and 7 times from pelvic abscesses whereas solely aerobes were isolated 14 times from superficial and 3 times from pelvic abscesses. This difference in distribution was statistically significant ($P < 0.01$; χ^2-test).

Table 1. Number and types of aerobic and anaerobic bacteria isolated from 101 abscesses.

Aerobic		Anaerobic	
Species	Number of isolates	Species	Number of isolates
Corynebacterium pyogenes	61	Fusobacterium necrophorum	45
Corynebacterium species	17	Fusobacterium russii	1
Streptococci serotypes C, P, L, U	16	Fusobacterium species	4
Streptococci species	9	Bacteroides ruminicola	14
Staphylococci aureus	4	Bacteroides oralis	5
Pasteurella multocida	3	Bacteroides bivius	2
Escherichia coli	3	Bacteroides ovatus	2
Miscellaneous[a]	7	Bacteroides melaninogenicus ss. melaninogenicus	1
Unidentified	3	Bacteroides uniformis	1
		Bacteroides asaccharolyticus	1
		Bacteroides vulgatus	1
		Bacteroides species	10
		Peptostreptococcus anaerobius	16
		Peptostreptococcus parvulus	1
		Peptococcus indolicus	14
		Peptococcus prevotii	4
		Peptococci species	3
		Anaerobic cocci	18
		Miscellaneous[b]	4
		Unidentified	8

[a] Including micrococci 5; Pasteurella sp. 1; Actinobacillus sp. 1.

[b] Including anaerobic streptococci 3; Bifidobacterium sp. 1.

A pure culture was obtained from 28 abscesses. C. pyogenes was isolated from 15 and F. necrophorum from 8 of these. From 73 abscesses between 2 and 7 different species were isolated. The most frequent combination of bacterial species found was C. pyogenes/F. necrophorum. This combination occurred exclusively in 8 and together with other bacteria in 15 abscesses. In all, 123 aerobic and 155 anaerobic bacteria were isolated. Numbers and types of isolated bacteria are summarized in Table 1.

The "Corynebacterium species" group consisted of Gram-positive rods. When grown on horse blood agar, colonies resembled C. pyogenes but were slightly larger. A few strains showed weak hemolytic activity after 24 h incubation and all were slightly to moderately hemolytic after 48 h incubation at 37°C. All strains produced acid from glucose, lactose, maltose, sucrose and

some from rhamnose. No strain produced gelatinase or urease. All but 1 strain produced acid in litmus milk. No strain hemagglutinated sheep or pig erythrocytes.

Hemagglutination titres for isolates of C. pyogenes are shown in Fig. 1. In 43 strains titres were higher with pig erythrocytes than with sheep erythrocytes. Median hemagglutination titres were 1:16 and 1:8 for pig and sheep erythrocytes, respectively.

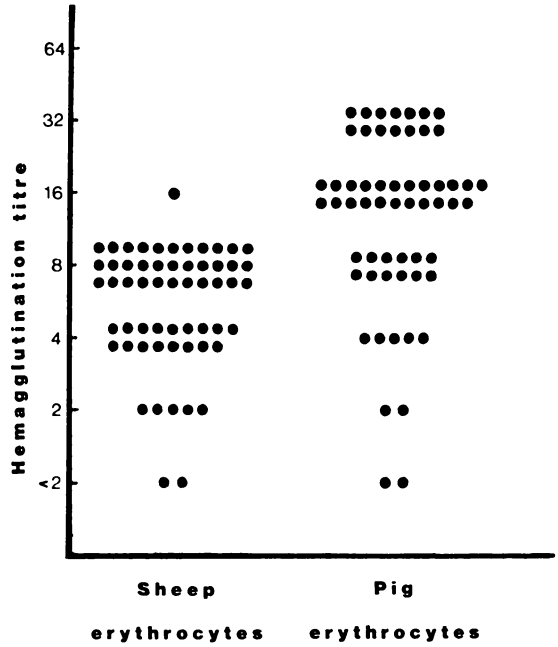

Figure 1. Hemagglutination titres of 58 strains of Corynebacterium pyogenes from pig abscesses on sheep erythrocytes and pig erythrocytes. Each solid circle represents 1 strain. Titres are expressed as reciprocals of highest dilutions giving $\gtrsim$ 100 % hemagglutination.

Three of 4 isolates of Staphylococcus aureus produced enterotoxin type A.

Of the anaerobes, F. necrophorum was the most common single species isolated.

A variety of anaerobic cocci were frequently isolated, especially Peptococcus anaerobius and P. indolicus. The biochemical non-reactiveness of many anaerobic cocci complicated their typing to species level.

The chromatogram of most non-speciated Bacteroides strains were similar to those of B. ruminicola but they differed biochemically.

DISCUSSION

Reports from many countries of the bacteriological composition of swine abscesses have been published as referred to in the introduction. A main feature is the reported high prevalence of C. pyogenes except in cervical lymphadenitis where streptococcus serogroup E is the most frequently isolated species (*Armstrong & Payne* 1969). However, the remaining aerobic and anaerobic flora diverges considerably in different reports. This is probably due to factors such as differences in abscess localisation, variations in management of pigs and differences in amount and kinds of antibiotics and growth promoting additives given, resulting in differences in the environmental and indigenous flora from which most infections emanate. Methods of sampling and cultivation of bacteria might also influence final isolation rates.

The role of C. pyogenes as a pathogen for animals is well established. The virulence factors responsible for the pathogenicity of C. pyogenes are less well understood. *Lindal & Smyth* (1982) described hemagglutinating capacity of C. pyogenes as a possible virulence factor by which C. pyogenes can stick to mammalian cells. They generally found higher hemagglutinating titres for sheep erythrocytes when using strains isolated from bovine mastitis. In the present study hemagglutinating titres were generally higher for pig erythrocytes than for sheep erythrocytes. The possibility of an agglutinin specificity of C. pyogenes strains to cells from different animal species merits further investigations.

The "Corynebacterium species" strains isolated in the present study most likely correspond to the atypical C. pyogenes strains described by *McCracken & McCaughey* (1973) and *Jones* (1980), and Corynebacterium pseudopyogenes described by *Hara et al.* (1976) and *Benno et al.* (1982). The taxonomic position of these Corynebacterium strains is not clear (*Buchanan & Gibbons* 1974). The lack of hemagglutinating activity is not surprising though, as few Corynebacterium species possess this ability (*Yanagava & Honda* 1976).

Of the streptococci, serotype C was most frequently isolated. Serogroup E streptococci were not isolated and it seems that this group preferentially is isolated from cervical lymphadenitis. The ecological nische of this group seems to be the tonsils rather than the skin or nose of pigs (*Jones* 1976).

The occurrence of enterotoxin producing strains of S. aureus in abscesses indicate a potential food poisoning risk. The proposal, by *Norval* (1968), to condemn carcases with abscesses containing S. aureus seems justified.

F. necrophorum is a recognised pathogenic bacteria which has been isolated both in pure and mixed cultures from infections in animals and man (*Langworth* 1977). It is part of the normal intestinal flora of pigs though apparently in small numbers (*Aalbæk* 1972, *Robinsson et al.* 1981), and infections occur probably via faecal contamination of wounds and bruises. Both *De Bruin & Jaartsveld* (1962) and *Norval* (1968) note F. necrophorum as a common isolate, at least from some types of pig abscesses. This is in agreement with the results of the present study, where F. necrophorum was found in 45 % of all cases, but diverges greatly from the reports by *Jones* (1980) and *Benno et al.* (1982) where F. necrophorum was found in 0 and ca. 10 %, respectively, of all abscesses examined. The reason(s) for this discrepancy is not clear, especially as localisation of abscesses from which samples were taken does not seem to differ much.

A high percentage of isolated Bacteroides species consisted of B. ruminicola which is in agreement with results presented by *Benno et al.* (1982). This species is normally sparsely isolated from infections in animals (*Berkhoff* 1978, *Prescott* 1979, *Hirsch et al.* 1979). As B. ruminicola constitutes a substantial part of the caecal (*Robinson et al.* 1981) and faecal flora (*Terada et al.* 1976) of pigs it is possible that infections emanating from the faecal flora may also involve B. ruminicola.

Most Peptococcus isolates in the present study consisted of P. indolicus. *Sørensen* (1978 b) found P. indolicus in almost half of all pig abscesses examined, while *Benno et al.* (1982) mostly isolated P. asaccharolyticus. However, the differentiation of P. indolicus from P. asaccharolyticus may give conflicting results depending on type of tests used (*Schwan* 1979 b).

The significant difference in distribution of pure culture of aerobes and anaerobes as regards superficial and pelvic abscesses is probably an expression of the composition of the opportunistic flora gaining entrance to the place of infection.

Most abscesses examined in the present investigation contained 2 or more bacterial species consisting mostly of 1 aerobic and 1 or several anaerobic species. This is characteristic of infections where anaerobes are involved and makes it difficult to evaluate

the etiological role of isolated bacteria. The finding, though, that almost 40 % of all isolates consisted of C. pyogenes and/or F. necrophorum and that 1 or both of these were found in 84 of 101 abscesses strongly indicates an etiological role for these bacteria.

ACKNOWLEDGEMENTS

We thank Prof. Marie-Louise Danielsson-Tham for performing the Staphylococcus enterotoxin tests, Ass. Prof. Olof Söderlind for help in identifying the aerobic isolates and Mrs. Helena Pilawa for technical assistance.

REFERENCES

Aalbæk, B.: Gram-negative anaerobes in the intectinal flora of pigs. Acta vet. scand. 1972, *13*, 228—237.

d'Aubert, S.: Batteriologia degli ascessi di suino. (Bacteriology of swine abscesses). Arch. Vet. Ital. 1981, *32*, 5—6.

Armstrong, C. H. & J. B. Payne: Bacteria recovered from swine affected with cervical Lymphadenitis (Jowl abscesses). Amer. J. vet. Res. 1969, *30*, 1607—1612.

Balows, A., R. M. DeHaan, V. R. Dowell & L. B. Ceuze: Anaerobic Bacteria: Role in Disease. Charles C. Thomas, Springfield, Ill. 1974, 655 pp.

Benno, Y., T. Mitsouka & S. Shirasaka: Anaerobic bacteria isolated from abscesses in pigs. Jap. J. vet. Sci. 1982, *44*, 309—315.

Berkhoff, G. A.: Recovery and identification of anaerobes in veterinary medicine: a 2-year expericence. Vet. Microbiol. 1978, *2*, 237—252.

Buchanan, R. E. & M. E. Gibbons: Bergey's Manual of Determinative Bacteriology, 8 ed. Williams & Wilkins Co, Baltimore, USA 1974.

De Bruin, J. J. M. & F. H. J. Jaartsveld: Staartafwijkingen bij varkens als oorzak voor het ontstaan van abscessen. (Abscesses caused by deviation of the tail in pigs). Tijdschr. Diergeneesk. 1962, *12*, 847—851.

Cowan, S. T.: In: Cowan & Steel: Manual for the Identification of Medical Bacteria, University Press, Cambridge 1977.

Hara, F., H. Veno, J. Okada & A. Iawata: Bacteriological and serological examination of pigs with pyogenic infections. J. Jap. vet. med. Ass. 1976, *29*, 73—77. (In Japanese).

Hirsch, D. H., E. L. Biberstein & S. S. Jang: Obligate anaerobes in clinical veterinary practice. J. clin. Microbiol. 1979, *10*, 188—191.

Holdemann, L. V., E. P. Cato & W. E. C. Moore: Anaerobe Laboratory Manual, 4 ed., VPI, Blacksburg, Virginia 1977.

Jones, J. E. T.: The carriage of beta-haemolytic streptococci by healthy pigs. Brit. vet. J. 1976, *132*, 276—283.

Jones, J. E. T.: Observation on the bacterial flora of abscesses in pigs. Brit. vet. J. 1980, *136*, 343—348.

Lancefield, R.: Serological differentation of human and other groups of hemolytic streptococci. J. exp. Med. 1933, *57*, 571—595.

Langworth, B. F.: Fusobacterium necrophorum: Its characteristic and role as an animal pathogen. Bacteriol. Rev. 1977, *41*, 373—390.

Lindal, E. & C. J. Smyth: Hemagglutinating activity in Corynebacterium pyogenes. Current Microbiol. 1982, 7, 113—118.

McCracken, A. & W. J. McCaughey: A survey of abscesses in bacon weight pig. Brit. vet. J. 1973, *129*, 359—361.

Norval, J.: Abscesses in pigs. Vet. Rec. 1966, *78*, 708—712.

Prescott, J. F.: Identification of some anaerobic bacteria in nonspecific infections in animals. Canad. J. comp. Med. 1979, *43*, 194—199.

Robinson, I. M., M. J. Allison & J. A. Bucklin: Characterization of the cecal bacteria of normal pigs. Appl. environm. Microbiol. 1981, *41*, 950—955.

Schwan, A.: Immune response to anaerobic bacteria. Antibody production in human infection and experimental studies with bacterioides fragilis. Thesis, Uppsala University 1981.

Schwan, O.: Heifermastitis and dry cow mastitis. Bacteriological and serological investigations with special reference to mixed infection with Corynebacterium pyogenes, Peptococcus indolicus and Microaerophilic cocci. Thesis, Swedish University of Agricultural Sciences 1979 a.

Schwan, O.: Biochemical, enzymatic and serological differentiation of Peptococcus indolicus (Christiansen) Sorensen from Peptococcus asaccharolyticus (Distaso) Douglas. J. clin. Microbiol. 1979 b, *9*, 157—162.

Sørensen, G. H.: Bacteriological examination of summermastitis secretion. The demonstration of Bacteroidaceae. Nord. Vet.-Med. 1978 a, *30*, 199—204.

Sørensen, G. H.: Studies on the occurrence of Peptococcus indolicus and Corynebacterium pyogenes in abscesses in swine, and on the occurrence of P. indolicus in apparently normal skin and mucous membranes of piglets. Nord. Vet.-Med. 1978 b, *30*, 282—285.

Terada, A., K. Uchida & T. Mitsuoka: Die Bacteroidacienflora in den Feces von Scheinen. (The Bacteroidaceae flora in the faeces of pigs). Zbl. Bakt. I. Abt. Orig. 1976, *234*, 362—370.

Yanagawa, R. & E. Honda: Presence of pili in species of human and animal parasites and pathogens of the genus Corynebacterium. Infect. Immun. 1976, *13*, 1293—1295.

Zehren, V. L. & V. F. Zehren: Examination of large quantities of cheese for Staphylococcal enterotoxin A. J. Dairy Sci. 1968, *51*, 635.

SAMMANFATTNING

Isolering och karaktärisering av bakterier från bölder från normalslaktade svin.

101 svinbölder från olika delar av slaktkroppen undersöktes med avseende på förekomst av aeroba och anaeroba bakterier. Växt av bakterier påvisades i samtliga bölder. I 68 bölder fanns en blandad aerob och anaerob flora, i 22 bölder enbart aerober och i 11 enbart anaerober. Corynebacterium pyogenes och Fusobacterium necrophorum återfanns i 61 respektive 45 bölder. C. pyogenes uppvisade högre hämagglutinationstitrar mot svin- än mot fårblodkroppar. Tre av 4 isolat av Staphylococcus aureus bildade enterotoxin typ A.

(Received December 23, 1982).

Reprints may be requested from: Anders Engvall, the Department of Food Hygiene, Faculty of Veterinary Medicine, Swedish University of Agricultural Sciences, S-750 07 Uppsala, Sweden.