ISOLATION OF OBLIGATE ANAEROBIC BACTERIA FROM BOVINE ABSCESES IN SITES OTHER THAN THE LIVER

M. KANOE, K. NOUKA AND M. TODA

Department of Veterinary Microbiology, Faculty of Agriculture, Yamaguchi University, Yamaguchi-shi, Yamaguchi 753, Japan

SUMMARY. A survey in Japan showed that of 2036 slaughtered cattle 58(3%) had abscesses in sites other than the liver. In 21 of the affected animals the lesions were pulmonary and in 32 abdominal (excluding hepatic); in five animals the lesions were found elsewhere (muscle 2, skin 2, bone 1). Nineteen (33%) of the 58 cattle also had abscesses in the liver.

Obligate anaerobes alone were isolated from 17(29%) of the affected animals (Fusobacterium necrophorum 14, Bacteroides spp. 2, Peptostreptococcus anaerobius 1). A mixture of obligate anaerobes and aerobes or facultative anaerobes was isolated from 31 affected cattle: of these animals 21 yielded large and five small numbers of F. necrophorum; three yielded fusobacteria other than F. necrophorum; and two yielded Propionibacterium acnes. The remaining 10 affected animals yielded only aerobes or facultative anaerobes. The numbers of viable obligate anaerobes in pus specimens were in the range 10^3–10^9/ml.

INTRODUCTION

Bovine visceral absceses are frequently found in slaughterhouses in Japan. Hepatic absceses have been well investigated bacteriologically and Fusobacterium necrophorum has long been recognised as their most important cause (Newsom, 1938; Yamamoto, 1938; Simon and Stovell, 1971; Kanoe et al., 1976). On the other hand, there are only a few reports (Berkhoff, 1978; Hirsh, Biberstein and Jang, 1979; Prescott, 1979) of the organisms found in bovine thoracic and abdominal (excluding hepatic) absceses, which are assumed to be caused by anaerobic bacterial infections.

More information on such absceses would be of direct interest to veterinarians and of comparative interest to medical bacteriologists. We therefore report the results of a study of the species and numbers of bacteria in absceses of bovine lung, abdominal viscera (other than liver), liver, bone, muscle and skin.

MATERIALS AND METHODS

Specimens. A total of 2036 cattle slaughtered at the Futsukaichi Abattoir in Fukuoka Prefecture, Japan, between Dec. 1980 and Nov. 1981 were examined. Fifty-eight absceses from different cattle were examined bacteriologically; the lesions were found in lung, lymph nodes,
kidneys, diaphragm, spleen, reticulum, rumen, bone, muscle and skin. Hepatic abscesses found in 19 of the 58 animals with the lesions mentioned above were also investigated bacteriologically. Pus and fluid specimens were collected aseptically from the abscesses, immediately placed into sterile stoppered tubes filled with O₂-free CO₂ (Holdeman, Cato and Moore, 1977), and transported to the laboratory in a chilled container.

**Isolation and enumeration of bacteria.** A 0.5-ml portion of each specimen was diluted tenfold with 4.5 ml of a solution, pH 7.2, containing KH₂PO₄ 0.45% w/v, Na₂HPO₄ 0.5% w/v, cysteine monohydrochloride 0.1% w/v and agar 0.1% w/v in distilled water. A 0.05-ml volume of each of four dilutions (10, 10², 10³, 10⁴) was spread on an agar plate. Cultures on blood agar (BA) consisting of Trypticase Soy Agar (BBL, Baltimore, MD, USA) with rabbit erythrocytes 5%, on Sabouraud Agar, and on DHL Agar (Eiken Chemical Co., Tokyo) were incubated aerobically at 37°C for 1-5 days. Other cultures on BA, on Modified FM Agar (Nissui Seiyaku Co., Tokyo) supplemented with rabbit erythrocytes 5%, and on Bacteroides Agar (Nissui Seiyaku Co., Tokyo) were incubated at 37°C for 5 days in a jar made anaerobic by the iron-wool method (Parker, 1955) and containing CO₂ 10%. Anaerobic cultures were also made on MI0 medium (Caldwell and Bryant, 1966), sterilised anaerobically and prerduced (Mitsuoka et al., 1969). After incubation the colonies were counted.

**Identification of cultures.** Identification of aerobic or facultative anaerobic isolates was by standard methods (Cowan, 1974).

Colonies growing anaerobically were subcultured on BA plates in duplicate to allow for aerobic and anaerobic incubation. Organisms growing only on anaerobic plates were accepted as obligate anaerobic bacteria and examined for their reaction in Gram's stain and colonial and cellular morphology. Obligate anaerobes were transferred to peptone yeast glucose broth (Holdeman et al., 1977) and cultured until maximum growth occurred. Extracts were then prepared and analysed for volatile and non-volatile fatty acids by the procedure of Holdeman et al. (1977) with a slight modification. The extracts were injected on to a 2.0 m x 4 mm glass column of Reoplex 10% or FAL-M 10% on Chromosorb W(60-80 mesh) obtained from Shimazu Ltd, Tokyo. The dual columns were operated in a model GC 7AG Gas Chromatograph (Shimazu Ltd, Tokyo) equipped with a Flame Ionisation Detector. The initial temperature was 130°C and injection temperature 220°C. The nitrogen gas pressure was 1 kg/cm². The pressures for air and hydrogen gas were 0.5 kg/cm² and 0.6 kg/cm², respectively. Resultant peaks were integrated and recorded by means of a model R-11 Integrator (Shimazu Ltd, Tokyo). Stock solutions of standard alcohols and acids were prepared as described by Holdeman et al. (1977).

The species of bacteria were identified by established criteria (Werner, 1974; Holdeman et al., 1977). Tests included observation of colonial and cellular morphology, reaction in Gram's stain, pigment formation, production of catalase, lactate utilisation, production of indole, growth stimulation or inhibition by bile, gelatin liquefaction, glutamine decarboxylase reaction, haemagglutination of chicken red-blood cells, lecithinase and lipase reactions, aesculin hydrolysis, and acid production from carbohydrates.

**Results**

**Occurrence of bovine abscesses**

Of 2036 cattle examined, 314 (15%) had abscesses in the liver; 19 had abscesses in the liver and in other sites; and 39 had abscesses only in sites other than the liver. Of the 58(3%) cattle with abscesses in sites other than the liver, 21 were affected in the lungs, 32 in abdominal viscera (other than liver), two in muscle, two in skin, and one in bone. As shown in the table, the incidence of hepatic lesions was higher in animals with abscesses in other abdominal viscera (44%) than in animals with abscesses of the lung (24%).

**Cultural examination of lung, abdominal (other than hepatic), bone, muscle and skin abscesses**

Specimens from the 58 cattle with lesions in sites other than the liver invariably...
TABLE

Incidence of hepatic lesions in animals with abscesses in other sites

<table>
<thead>
<tr>
<th>Animals (number) with abscesses in</th>
<th>Number (%) of animals that also had hepatic lesions</th>
</tr>
</thead>
<tbody>
<tr>
<td>lung (21)</td>
<td>5 (24)</td>
</tr>
<tr>
<td>abdominal lymph node (13)</td>
<td>3</td>
</tr>
<tr>
<td>diaphragm (7)</td>
<td>5</td>
</tr>
<tr>
<td>kidney (9)</td>
<td>4</td>
</tr>
<tr>
<td>spleen (1)</td>
<td>1</td>
</tr>
<tr>
<td>reticulum (1)</td>
<td>1</td>
</tr>
<tr>
<td>rumen (1)</td>
<td>0</td>
</tr>
<tr>
<td>bone (1)</td>
<td>0</td>
</tr>
<tr>
<td>muscle (2)</td>
<td>0</td>
</tr>
<tr>
<td>skin (2)</td>
<td>0</td>
</tr>
<tr>
<td>Total 58</td>
<td>19 (33)</td>
</tr>
</tbody>
</table>

contained bacteria in excess of $10^5$ cfu/ml. Seventeen (29%) specimens contained obligate anaerobes only, in pure culture. They were: *F. necrophorum* (14 abscesses), *Bacteroides* spp. (2), and *Peptostreptococcus anaerobius* (1).

Thirty-one (53%) specimens yielded both obligate anaerobes and aerobes or facultative anaerobes. *F. necrophorum* was predominant in 21 specimens, and was also present, though not abundantly, in a further five. In these 26 pus samples, *F. necrophorum* was associated with either two or four other bacterial species, namely, *Corynebacterium pyogenes* (15 abscesses), *Corynebacterium* spp. (7), *Staphylococcus epidermidis* (6), *Escherichia coli* (6), gram-negative aerobic rods (6), *Pasteurella multocida* (5), *Streptococcus zooepidemicus* (4), *B. fragilis* group (3), gram-positive aerobic rods (2), *F. mortiferum* (1), *S. aureus* (1), *C. equi* (1) and *Actinobacillus lignieresi* (1). Three of the 31 abscesses contained fusobacteria other than *F. necrophorum*, and *C. pyogenes*; and two yielded *Propionibacterium acnes* and *S. epidermidis*.

Ten abscesses (17%) contained aerobes or facultative anaerobes only. The isolates were: *C. pyogenes* (4 abscesses), *P. multocida* (3), *A. lignieresi* (2), and *S. aureus* (1). *A. lignieresi* was derived from lung abscesses and *P. multocida* from lesions in the lymph nodes.

The ranges (means) for the viable counts (cfu/ml) were as follows: *F. necrophorum*, $10^3-10^9$ (10^6); *B. fragilis* group, $10^6-10^8$ (10^7); fusobacteria other than *F. necrophorum*, $10^4-10^6$ (10^5); *Bacteroides* spp., $10^7-10^9$ (10^8); *Pept. anaerobius*, 10^8; *F. mortiferum* and *Prop. acnes*, not estimated; *C. pyogenes*, $10^6-10^8$ (10^6); *S. epidermidis*, $10^4-10^6$ (10^5); *P. multocida*, $10^5-10^6$ (10^6); *Corynebacterium* spp., $10^3-10^6$ (10^5); *E. coli*, $10^4-10^7$ (10^5); gram-negative aerobic rods, $10^3-10^7$ (10^5); *Str. bovis*, $10^6-10^7$ (10^6); *Str. zooepidemicus*, $10^6$; *S. aureus*, $10^5$; gram-positive aerobic rods, $10^4$; and *A. lignieresi* and *C. equi*, not estimated.

Cultural examination of hepatic abscesses

Hepatic abscesses from the 19 cattle that also had abscesses in other sites invariably contained *F. necrophorum*. In nine instances the organism was found in pure culture. In the remainder it was associated with two or three of the following species: *C. pyogenes*
(8 abscesses), S. epidermidis (6), Str. uberis (6), Str. zooepidemicus (4), Corynebacterium spp. (4), gram-negative aerobic rods (3), Bacteroides spp. (2), and E. coli (2).

The ranges (means) for viable counts (cfu/ml) of the isolates were as follows: F. necrophorum, 10^5–10^8 (10^6); Bacteroides spp., 10^6–10^7 (10^6); C. pyogenes, 10^4–10^8 (10^6); Str. uberis, 10^5–10^7 (10^5); S. epidermidis, 10^4–10^6 (10^5) Str. zooepidemicus, 10^5–10^6 (10^5); Corynebacterium spp., 10^5–10^7 (10^5); gram-negative aerobic rods, 10^4–10^6 (10^4); and E. coli, 10^3–10^5 (10^4).

**DISCUSSION**

In this study, the incidence of abscesses in bovine lung, abdominal viscera (other than liver), muscle, bone and skin was generally lower than that in the liver. F. necrophorum was recovered in pure culture or was dominant in most of the lung, abdominal (other than hepatic) and hepatic abscesses examined. Mean viable counts of this species were high. The second most common isolate was C. pyogenes, which had high counts in some specimens.

It is well known that F. necrophorum is a pathogen commonly found in domestic and wild animals and frequently isolated from various lesions as a secondary invader (Simon and Stovell, 1969; Langworth, 1977). Moreover, it is considered to be a member of the intestinal flora of healthy herbivores and rabbits (Koosaka, 1974; Kanoe, Izuchi and Toda, 1978). Insufficient attention has been paid to the mechanisms of infection with this species. It is not clear, for example, how F. necrophorum in the intestinal tract finds its way into visceral organs to produce lesions. The organism’s virulence factors are also poorly understood. These problems are under investigation.

We wish to thank the staff officers of the Futsukaichi Health Center, Fukuoka Prefecture, for cooperation in collecting samples.

**REFERENCES**


ANAEROBIC BACTERIA IN BOVINE ABSCESSES