Hepatic Abscess Formation in Cattle Inoculated with
Fusobacterium necrophorum

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Viable cells of Fusobacterium necrophorum biovar A were inoculated into the ruminal vein of cattle. Two cattle inoculated with more than $4.4 \times 10^8$ viable cells died within 2 days after inoculation. Disseminated necrotic foci were observed in their liver. When $2.8 \times 10^6$ to $1.4 \times 10^8$ viable cells were inoculated into 7 cattle, these cattle manifested no sickness clinically, with the exception of one calf which died. By autopsy, however, hepatic abscesses were recognized in these cattle 9 to 21 days after inoculation. In addition, lung abscesses were also formed in 3 calves of 2 months old. F. necrophorum was recovered from the hepatic abscesses and lung abscesses in viable counts ranging from $10^6$ to $10^7$ per gram. The organism were also isolated from the normal position of the liver, the hepatic lymph node and other organs in some cattle. But the viable counts were lower than those of the abscesses. In two calves, Corynebacterium pyogenes was isolated from the hepatic abscess and lymph node. On the contrary, no hepatic abscess nor necrotic focus was formed in a calf when $1.2 \times 10^8$ viable cells were inoculated into the jugular vein. The precipitating antibody was demonstrated in all cattle having hepatic abscesses by the immunodiffusion test with the concentrated culture supernatant of F. necrophorum. The maximum antibody titer in the cattle ranged from 16 to 128.---Key words: Bovine, Fusobacterium necrophorum, Hepatic abscess.

Hepatic abscess is frequently detected in barley beef cattle, feedlot beef cattle and fattened dairy steers in association with ruminal lesions such as ruminitis and rumen parakeratitis [8, 16, 19]. Fusobacterium necrophorum is the primary etiologic agent of hepatic abscess and a component of the normal rumen flora [1, 2, 4, 10, 11, 13, 15, 20]. Kanoe et al. [11] demonstrated that F. necrophorum is also isolated from lesions of the ruminal wall and from reticular abscesses. Therefore, it has been considered that the ruminal lesions are the primary foci of the ruminitis-hepatic abscess complex, and that hepatic abscesses are the secondary foci of the infection.

On the other hand, the experimental infection of F. necrophorum in cattle has been attempted by some investigators. Robinson et al. [13] inoculated a viable culture of F. necrophorum into the jugular vein of cattle, but no hepatic abscess developed in the cattle. In 1954, Jensen et al. [9] were able to produce hepatic abscesses in cattle and sheep by intraportal inoculation of F. necrophorum. More recently, Scanlan and Berg [14] also produced hepatic abscesses in cattle experimentally by intraportal inoculation. These previous reports, however, gave little information on bacteriological and serological examination, especially on the number of inoculated and recovered organisms. In addition, a difficult abdominal leparotomy is necessary for intraportal inoculation.

Therefore, in the present study, the authors inoculated viable cells of F. necrophorum

into the ruminal vein of cattle. This paper describes the hepatic abscess formation and bacteriological and serological findings in cattle inoculated with *F. necrophorum* biovar A. Details of the pathological study of the inoculated cattle are reported elsewhere.

**MATERIALS AND METHODS**

*Experimental animals:* Ten cattle were used as experimental animals in the present experiments. Their ages varied from 2 months to 6 years old, and the breeds were Holstein and Japanese Black Cattle. They were numbered from 1 to 10 in the order of experimental inoculation.

*Organisms and cultivation:* *F. necrophorum* strain ATCC 25286, originated from the American Type Culture Collection, was used throughout the present experiments. The organisms were inoculated into modified VL broth [18] and cultivated anaerobically at 37°C for 18 hr by the CO₂ gas jet method, which was modified by Azuma and Suto [2] from the original description of Hungate [7]. They were harvested by centrifugation and suspended in cold modified VL broth for the animal inoculation. The viable count of the resulting suspension was determined by the roll tube method with modified VL agar [2, 7].

*Animal inoculation procedure:* Each cattle was sedated by an intramuscular injection of 2% Celactal injectable solution (Bayer, West Germany). The abdominal incision site, the intermediate position between the 13th costa and the hipborn, was anaesthetized locally with Procaine injectable solution (Fujita, Japan). Then the site, which was about 10 cm in length, was incised surgically in the order of skin, abdominal muscles and peritoneum. From the incision hole, a part of the rumen was drawn out carefully, and the ruminal vein was inoculated with 10 ml of the bacterial suspension. After inoculation, the incision site was sutured surgically. The operation time was about 30 to 40 min.

*Autopsy of cattle:* The cattle were autopsied at various intervals after inoculation or immediately after death. The internal organs and hepatic lymph node were examined macroscopically for abscess formation and collected for bacteriological examination.

*Bacteriological examination:* The internal organs and lymph node were weighed and triturated separately in glass homogenizers containing 5 ml of sterile saline. The resulting samples were serially diluted tenfold with sterile saline. One-tenth ml volumes of diluted samples were added to modified VL agar and cultivated at 37°C for 2 days. After counting of the bacterial colonies, the isolated organisms were examined by Gram's staining. *F. necrophorum* was confirmed by fluorescent antibody staining.

*Serological examination:* Sera were collected from some cattle at various intervals after inoculation and examined for the appearance of antibody to *F. necrophorum* by the immunodiffusion test [17]. In the test, lipopolysaccharide (LPS), cytoplasmic toxin, and concentrated culture supernatant of *F. necrophorum* were used as antigens. LPS and cytoplasmic toxin were prepared by the procedures of Garcia *et al.* [5, 6]. The culture supernatant was concentrated to 1/50 volume with ultrafiltration using XM 300 membrane (Amicon).

**RESULTS**

1. Hepatic abscess formation in cattle inoculated with *F. necrophorum*.

Ten cattle were inoculated with the viable cells of *F. necrophorum* and examined for hepatic abscess formation. The results obtained are shown in Table 1.

In first experiment, 10 ml of the bacterial suspension containing 1.2×10⁹ viable cells per ml were inoculated into the jugular vein of calf No. 1. The calf was killed for autopsy after 2 days inoculation; however, no hepatic lesions were recognized.

Therefore, the inoculating route using the
Table 1. Hepatic abscess formation in cattle inoculated with E. necrophorum

<table>
<thead>
<tr>
<th>Number</th>
<th>Age</th>
<th>Route</th>
<th>Organisms CFU/ml</th>
<th>Days from inoculation to autopsy</th>
<th>Gross lesion</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1 weeks</td>
<td>Jugular vein</td>
<td>$1.2 \times 10^9$</td>
<td>2</td>
<td>No lesion</td>
</tr>
<tr>
<td>2</td>
<td>1 year</td>
<td>Ruminal vein</td>
<td>$1.7 \times 10^{10}$</td>
<td>1 (D)*</td>
<td>Necrotic foci in liver</td>
</tr>
<tr>
<td>3</td>
<td>1 year</td>
<td>Ruminal vein</td>
<td>$4.4 \times 10^8$</td>
<td>2 (D)</td>
<td>Necrotic foci in liver</td>
</tr>
<tr>
<td>4</td>
<td>10 months</td>
<td>Ruminal vein</td>
<td>$6.1 \times 10^7$</td>
<td>9</td>
<td>Abscesses in liver</td>
</tr>
<tr>
<td>5</td>
<td>6 years</td>
<td>Ruminal vein</td>
<td>$1.9 \times 10^7$</td>
<td>14</td>
<td>Abscesses in liver</td>
</tr>
<tr>
<td>6</td>
<td>2 months</td>
<td>Ruminal vein</td>
<td>$1.4 \times 10^8$</td>
<td>21</td>
<td>Abscesses in liver, lung</td>
</tr>
<tr>
<td>7</td>
<td>2 months</td>
<td>Ruminal vein</td>
<td>$7.1 \times 10^7$</td>
<td>21</td>
<td>Abscesses in liver, lung</td>
</tr>
<tr>
<td>8</td>
<td>2 months</td>
<td>Ruminal vein</td>
<td>$5.2 \times 10^7$</td>
<td>4 (D)</td>
<td>Necrotic foci in liver, lung</td>
</tr>
<tr>
<td>9</td>
<td>2 months</td>
<td>Ruminal vein</td>
<td>$8.4 \times 10^6$</td>
<td>21</td>
<td>Abscesses in liver, lung</td>
</tr>
<tr>
<td>10</td>
<td>2 months</td>
<td>Ruminal vein</td>
<td>$2.8 \times 10^6$</td>
<td>21</td>
<td>No lesion</td>
</tr>
</tbody>
</table>

* Cattle died.

ruminal vein, described in methods, was used in all subsequent experiments. Cattle Nos. 2 and 3, aged one year, died within 1 to 2 days when 10 ml of the bacterial suspension containing $1.7 \times 10^{10}$ or $4.4 \times 10^8$ viable cells per ml were inoculated into the ruminal vein, respectively. By autopsy, a mass of necrotic foci from 2 to 3 mm in diameter was observed in their liver. In addition, remarkable hyperemia and hemorrhages were also recognized in the rumen, heart, and lung.

Next, cattle Nos. 4 and 5, aged 10 months and 6 years, were inoculated with 10 ml of the bacterial suspensions containing $6.1 \times 10^7$ or $1.9 \times 10^7$ viable cells per ml and autopsied 9 or 14 days later, respectively. Three hepatic abscesses from 1 to 3 cm in diameter were recognized in both cattle, but no macroscopical lesions were observed in the other organs.

Calves Nos. 6, 7, 8, 9 and 10, aged 2 months, were inoculated with the bacterial suspensions containing $2.8 \times 10^6$ to $1.4 \times 10^8$ viable cells per ml. Out of these, one calf (No. 8) died with parietal hernia 4 days after inoculation. A mass of necrotic foci was observed in the liver and lung of this calf. The remaining 4 calves were killed for autopsy 3 weeks after inoculation. In calves Nos. 6, 7 and 9, hepatic abscesses and lung abscesses were found. The hepatic abscess size ranged from 1 to 10 cm in diameter. However, no hepatic abscess was observed in calf No. 10, which was inoculated with the bacterial suspension containing $2.8 \times 10^6$ viable cells per ml.

2. Recovery of organisms from inoculated cattle.

An attempt was made to recover the inoculated organisms quantitatively from the internal organs and lymph node of the inoculated cattle. The results obtained are shown in Table 2.

In calves Nos. 1 and 10, which showed no lesions, no organisms were recovered from the tested organs and the lymph node. On the contrary, a population of $10^8$ viable cells of F. necrophorum was present in the livers containing necrotic foci of the cattle which died, Nos. 2 and 3. In the case of cattle Nos. 4 and 5, the organisms were recovered from the abscess portion of the livers in viable counts ranging from $10^6$ to $10^8$ per gram. It was also recovered from the normal position of the livers and hepatic lymph node, although the viable counts were lower than those of the abscesses. In addition, Corynebacterium pyogenes was isolated from the hepatic lymph node of cattle No. 4. In the case of calves Nos. 6, 7, and 9, hepatic abscesses and lung abscesses were recognized.
Table 2. Recovery of organisms from infected cattle

<table>
<thead>
<tr>
<th>Cattle number</th>
<th>No. 1</th>
<th>No. 2</th>
<th>No. 3</th>
<th>No. 4</th>
<th>No. 5</th>
<th>No. 6</th>
<th>No. 7</th>
<th>No. 8</th>
<th>No. 9</th>
<th>No. 10</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cerebrum</td>
<td>NT*</td>
<td>NT</td>
<td>—</td>
<td>—</td>
<td>NT</td>
<td>—</td>
<td>NT</td>
<td>—</td>
<td>NT</td>
<td>—</td>
</tr>
<tr>
<td>Heart</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>4.66**</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Lung (Normal)</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>3.77</td>
<td>6.59</td>
<td>8.20</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>(Abscess, Necrosis)</td>
<td>.</td>
<td>.</td>
<td>.</td>
<td>.</td>
<td>8.63</td>
<td>8.61</td>
<td>9.04</td>
<td>8.30</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Liver (Normal)</td>
<td>—</td>
<td>.</td>
<td>3.78</td>
<td>3.78</td>
<td>—</td>
<td>5.27</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>(Necrotic foci)</td>
<td>8.51</td>
<td>8.11</td>
<td>.</td>
<td>.</td>
<td>.</td>
<td>9.07</td>
<td>.</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>(Abscess 1)</td>
<td>—</td>
<td>.</td>
<td>8.88</td>
<td>8.79</td>
<td>7.66</td>
<td>7.23</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>(Abscess 2)</td>
<td>—</td>
<td>.</td>
<td>8.43</td>
<td>8.11</td>
<td>8.57</td>
<td>8.69</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>(Abscess 3)</td>
<td>—</td>
<td>.</td>
<td>6.61</td>
<td>6.34</td>
<td>.</td>
<td>8.83</td>
<td>.</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Spleen</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Kidney</td>
<td>—</td>
<td>3.51</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Portal fissure lymph node</td>
<td>—</td>
<td>—</td>
<td>3.64</td>
<td>2.97</td>
<td>4.20</td>
<td>4.54</td>
<td>5.86</td>
<td>NT</td>
<td>2.77</td>
<td>—</td>
</tr>
</tbody>
</table>

*: Not tested.
**: Log number of organisms per gram of abscess or tissue.

Dashes indicate that the bacterial counts were less than $10^8$ per gram.

3 weeks after inoculation. *F. necrophorum* was recovered from these abscesses at the levels of $10^7$ to $10^9$ viable cells per gram. In addition, *C. pyogenes* was also isolated from the hepatic abscess of calf No. 6. *F. necrophorum* was recovered in pure culture from the liver, lung, heart, spleen and kidney of calf No. 8.

3. Antibody responses in infected cattle

Antibody responses to *F. necrophorum* in the infected calves Nos. 6, 7, 9 and 10 were examined by the immunodiffusion test.

When LPS and cytoplasmic toxin were used as an antigen, no precipitating antibody was demonstrated in sera from all the calves, except for calf No. 7. In calf No. 7, the precipitating antibody to LPS was demonstrated, but its titer was low (titer: 2). On the contrary, the precipitating antibody was demonstrated in all the calves having hepatic abscesses when the concentrated culture supernatant was used as an antigen. As shown in Fig. 1, the precipitating antibody titers began to rise 10 to 13 days after inoculation. The maximum titer ranged from 16 to 128.

**DISCUSSION**

It is generally believed that the pathogenesis of bovine hepatic abscess is the invasion of *F. necrophorum* into the deeper layers of the rumen wall from the ruminal lesions and then its entrance into the liver via the hepatic portal venous system, where it forms hepatic abscesses. In the present experiment, therefore, *F. necrophorum* was inoculated into the ruminal vein of cattle. As a result, hepatic abscesses or necrotic foci were formed in 8
out of 9 cattle inoculated with 10 ml of the bacterial suspensions containing $2.8 \times 10^6$ to $1.7 \times 10^{10}$ viable cells per ml. On the contrary, no hepatic abscess was observed when the organisms were inoculated into the jugular vein of the cattle. These results provided considerable evidence to justify the conjecture of the pathogenic route described above. In addition, the procedure of ruminal vein inoculation adopted in this study proved to be easier to perform than the hepatic portal vein inoculation described by Jensen et al. [9], and more efficient for the reproduction of hepatic abscess in cattle.

Jensen et al. [9] and Scanlan and Berg [14] reported that hepatic abscesses were produced in cattle with intraportal inoculation of F. necrophorum cultures. But the bacterial number of the inocula was not examined in their experiments. In the present experiment, hepatic abscesses were formed in the cattle inoculated with bacterial suspensions containing more than $8.4 \times 10^6$ viable cells, but not in the cattle inoculated with $2.8 \times 10^6$ viable cells. That is, although the organisms were inoculated into the ruminal vein of the cattle, inoculation of a large number of organisms was necessary in order to produce the hepatic lesions experimentally. In natural infection, even if a small number of organisms invade, there seems to be an environment in which the organisms can multiply and produce hepatic abscess. Therefore, it is necessary to examine the conditions for multiplication of the organisms. The authors conjecture that rumen acidosis or absorption of LPS from rumen bacteria play some role in the multiplication of the organisms.

In the natural cases of abscess formation, F. necrophorum was present in concentrations ranging from $10^4$ to $10^9$ viable cells per gram of the hepatic abscesses [3, 10]. The organisms were sometimes found with other bacteria, such as C. pyogenes and Bacteroides spp., in 10 to 61% of the abscesses. In the present experiment, F. necrophorum was recovered from the hepatic abscesses of the inoculated cattle in viable counts ranging from $10^6$ to $10^9$ per gram. Also, C. pyogenes was isolated from the hepatic lymph node or hepatic abscess. In the previous report [18], it was shown that C. pyogenes multiplied actively in hepatic abscesses of mice in which F. necrophorum had multiplied. From the previous result, it is thus conjectured that C. pyogenes, which lurks in the bovine body, invaded the hepatic abscess and multiplied by the support of F. necrophorum.

On the other hand, Kanoe and Toda [12] reported that antibody was detected in sera from cattle affected with hepatic abscess by the immunodiffusion test with HCl-extract of F. necrophorum. In addition, the precipitating antibody was developed in rabbits inoculated experimentally with the organisms. In the present experiment, antibody responses in the infected cattle were examined by the immunodiffusion test with LPS, cytoplasmic toxin and the concentrated culture supernatant of F. necrophorum. As a result, the precipitator antibody was demonstrated in the infected cattle having hepatic abscesses when the concentrated culture supernatant was used as an antigen. This result indicated that the determination of precipitating antibody may be useful for the diagnosis of F. necrophorum infection in cattle.

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REFERENCES
要約

Fusobacterium necrophorum を接種した牛における肝臓病の形成: 竹内正太郎・中島靖之1)・上田久1)・元井直之1)・小林良則・丙角雅雄(農林水産省家畜衛生試験場・北陸支場, 1)東北支場, 2)研究第 4 部)——牛の肝臓病の発症機序を解析する目的で, Fusobacterium necrophorum biovar A の生を

1) 竹内正太郎
2) 上田久
3) 元井直之
4) 小林良則
5) 丙角雅雄

Fusobacterium necrophorum を接種した 2 頭の牛は接種 2 日以内に死亡し, これらの肝臓には多核の壞死巣が認められた。これに対して, 2.8〜10 倍/ml の生を接種した 7 頭の牛では, 死亡した 1 頭の牛を除いて, 臨床的に異常を認めなかった。しかし, 接種 9〜21 日後の剖検時に肝臓病がこれらの牛で認められ, さらに, 2 カ月齢の牛では肝臓病も形成されていた。接種後の F. necrophorum は肝臓および肺臓病から 10^6〜10^7 倍/g 回収されたほか, 肝臓の正常部位, 肝門リンパ節および他の臓器からも分離された。接種前には, Coryne-

1) 竹内正太郎
2) 上田久
3) 元井直之
4) 小林良則
5) 丙角雅雄

たん天胞と沈降反応によって, 16〜128 倍の沈降抗体が肝臓病を有する牛で証明された。