Serum Sialic Acid Levels in Cattle Inoculated with *Fusobacterium necrophorum*

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ABSTRACT. *Fusobacterium necrophorum* was inoculated into the portal vein of seven cattle. Multiple necrotic foci were found in the livers of the two cattle which died on 10th and 14th day after inoculation. The remaining cattle autopsied on 12th to 126th day after inoculation were affected with hepatic abscesses. Some of them were affected also with extrahepatic abscesses found mainly in the lung and intercostal space. Serum sialic acid concentration was not elevated even 10 days after the inoculation in one of the two cattle affected with the multiple necrotic lesions in the liver. In the second it began to rise 8 days after the inoculation. In cattle affected with the hepatic abscesses, it began to rise above preinoculation values 3 to 5 days and reached a peak values 7 to 9 days after the inoculation. They remained at this level for 2 or 3 days and then either decreased gradually toward preinoculation values or remained somewhat elevated for more than 4 months. The effects of both hepatic and extrahepatic abscesses on the serum sialic acid level were discussed. Hepatic abscess formation correlated significantly with the rise in serum sialic acid level. —KEY WORDS: cattle, hepatic abscess, sialic acid.

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Determination of sialic acid or mucoprotein levels in blood may allow antemortem diagnosis of the acute phase of hepatic abscess in cattle [3]. These clinico-chemical indices, however, are not specific for hepatic abscess [3]. Determination of precipitating antibody may be useful for the diagnosis of *Fusobacterium necrophorum* infection [10]. Bovine hepatic abscess is only one pattern of the infection. We do not know which of these two measurements, sialic acid or antibody, is the better tool for diagnosis of the bovine hepatic abscess. Hepatic abscesses may also be imaged by ultrasonography, and such an examination was made on the cattle used in the present study and was reported in the preceding paper [2].

Plasma sialic acid and antibody titer against *F. necrophorum* have previously been determined in cattle inoculated with *F. necrophorum* into the portal vein or relating vessels following laparotomy [4, 10]. These

cattle were however affected not only by hepatic abscess but also extrahepatic ones and so were responding not only to the hepatic abscess but to a range of abscesses caused by *F. necrophorum* infection [10].

The cattle employed in the present study were affected with hepatic abscess and/or with extrahepatic abscesses, induced experimentally. We examined the effect of each form of abscess on blood sialic acid level and the antibody titer.

MATERIALS AND METHODS

Animals: Nine Holstein cattle were employed (Table 1). A *F. necrophorum* suspension was inoculated into the portal vein and abdominal wall to induce abscesses in the liver and abdominal wall, respectively. Preparation of the bacterial suspension and the inoculation procedure were noted in the previous paper [2].

Abscess induction in the abdominal wall:

Fig. 1. Effects of *F. necrophorum* inoculation into portal vein or abdominal wall on rectal temperature. Upper and middle charts: Left and right arrows indicate sterile broth or saline injection, and bacterial inoculation into portal vein, respectively. Middle chart: Not all data of Nos. 4 to 8 are plotted. They scattered around 39°C line before bacterial inoculation. Lower chart: Left arrow indicates bacterial inoculation into abdominal wall, and right one indicates surgical manipulation similar to that performed at left arrow with no bacterial inoculation.

A vertical incision 10cm in length was made under local anesthesia on the left side of the abdominal wall at about the position of the laparotomy incision employed by Takeuchi et al. [10] for inoculation of *F. necrophorum* into the ruminal vein. This was immediately followed by suturing the incision without manipulating the internal organs. After closing the peritoneum, 4 drops of the bacterial suspension were inoculated on the cut surfaces. In other respects standard aseptic technique was employed during these procedures. The only drugs employed were those used for sedation, local anesthesia and cutaneous disinfection.

Blood sialic acid: The concentrations in

the jugular blood were determined by a modification of the direct Ehrlich reaction [12] or by an enzyme testing kit (Kyokuto Pharmaceutical Co. Ltd., Tokyo) [11]. The Ehrlich's method was modified as follows. A mixture of 4.9 ml of water and 0.1 ml of serum in a test tube with an aluminium cap was heated for 5 min on a boiling water-bath, and then cooled in running water. One ml of the Ehrlich reagent was added to and mixed with the cooled solution, which was followed by the same procedure as the original method [12].

The concentration of sialic acid in a standard bovine serum stored at -20°C and used for this modification was 80 mg/dl. It

Table 1. Experimental animals, bacterial inoculation and pathological lesions detected

Cattle ^{a)}			<i>F. necrophorum</i> inoculation		Days from inoculation to autopsy	Hepatic abscess		Extrahepatic abscess (No.×size in cm)		Dermatitis at right breast
No	Sex & Body Weight (kg)	Cfu/ml	Volume (ml)	Number		Size (cm)	Lung	Intercostal space		
2	♂ 250	5.2×10 ⁷	5.0	10	hundreds	1– 2	+(1×1)	—	—	
3	♂ 264	9.3×10 ⁸	5.0	14	hundreds	1– 2	+(4×1)	+(1×5)	—	
4	♀ 296	1.8×10 ⁹	5.0	12	7	2– 5	—	—	—	
5	♀ 200	1.8×10 ⁹	3.0	55	20 ^{b)}	2–13	+(1×3)	—	—	
6	♀ 184	1.8×10 ⁹	1.5	56	1	5	—	+(1×4)	—	
7	♀ 197	3.4×10 ⁹	1.0	97	1	5	—	—	+ ^{c)}	
8	♂ 232	5.2×10 ⁷	4.0	126	3 ^{b)}	2– 5	—	—	+ ^{c)}	
Abdominal wall										
9	♂ 340	1.9×10 ⁹	4 drops into		0		—	+(1×12)	—	
10	♂ 310	1.9×10 ⁹	abdominal wall		0		—	+(1×12)	—	

a) Holstein breed.

b) Some of ultrasonographic lesions in the liver disappeared before autopsy.

c) Dermatitis was apparent for a period of the 25th to 40th day after inoculation.

was determined by the enzyme-testing kit. A standard straight line was obtained using 0.05, 0.1 and 0.15 ml of it. Measurements were made in duplicate.

Antibody: The titer of antibody against *F. necrophorum* was determined by the immunodiffusion test [9].

RESULTS

All the animals employed were first infused with 5 ml of sterile saline or broth into the portal vein. Neither pyrexia nor elevation of sialic acid level or antibody titer against *F. necrophorum* was observed following the infusion (Figs. 1 to 5).

Cattle Nos. 2 and 3 lost their appetite and suffered from pyrexia followed by hypothermia after the bacterial inoculation (Fig. 1) and had multiple necrotic lesions in their livers. An abscess 1 cm in diameter and 2 abscesses 3 mm in diameter were found in the lung and tonsile, respectively, in cattle No. 2. Four abscesses 1cm in diameter and an abscess 5 cm in diameter were seen in the lung and intercostal space, respectively, in cattle No. 3 (Table 1) Serum sialic acid

Fig. 2. Effects of sterile saline or broth injection (left arrows) and bacterial inoculation (right arrows) into portal vein on serum sialic acid concentration.

level of No. 2 had been higher than those of the other animals during the preinoculation period (Fig. 2). It was not elevated after the bacterial inoculation before death in cattle No. 2 but in No. 3 the level began to rise on the 8th day after inoculation.

Antibody titer to *F. necrophorum* began to rise 7 to 10 days after the bacterial inoculation. The maximum titer was 128 (Fig. 3).

In 5 cattle, Nos. 4 to 8, appetite was unaffected after the bacterial inoculations and the rectal temperature, although raised

Fig. 3. Effects of sterile saline or broth injection (left arrow) and bacterial inoculation (right arrow) on antibody titer against *F. necrophorum*.

after the inoculation, returned to normal within 2 to 10 days (Fig. 1). They had single or multiple abscesses in the liver (Table 1).

Cattle No. 5 had an abscess 3 cm in diameter in the lung, and cattle No. 6 had an abscess 4 cm in diameter in the intercostal space. This abscess had been seen in association with the hepatic abscess.

For a period between the 25th to 40th day after inoculation, cattle Nos. 7 and 8 were suffering from intraepidermal vesiculopustular dermatitis on the right body surface. It was caused by frequent application of the sound media, liquid paraffin, on the scanning area, and healed with an ointment (Exskin, Nissan-gohsei-Kogyo, Tokyo).

Levels of serum sialic acid began to rise above preinoculation values 3 to 5 days and reached peak values 7 to 9 days after the bacterial inoculations in cattle Nos. 4 to 8 (Fig. 4). The peak values were 15 to 30 mg/dl higher than the preinoculation levels. They remained at this level for 2 or 3 days and then decreased gradually toward preinoculation values except the case of cattle No. 8 (Fig. 4e).

The raised level in cattle No. 5 (Fig. 4b) did not return to the preinoculation level within 55 days.

In cattle No. 6 (Fig. 4c), the raised level returned to the preinoculation level on around the 30th day of postinoculation.

The curve of sialic acid in cattle No. 7

Fig. 4. Effects of sterile saline or broth injection (left arrows) and bacterial inoculation (right arrows) into portal vein on serum sialic acid concentration. Sialic acid concentrations in plasma were determined by enzyme-testing kit in (a) and (d), in which arrows indicate bacterial inoculation.

(Fig. 4d) showed two peaks. Afterwards it declined to the preinoculation level on around the 85th day after inoculation. The peak value of sialic acid of cattle No. 8 (Fig. 4e) persisted for a much longer period up to the end of experiment.

Antibody titer against *F. necrophorum* was examined in cattle Nos. 5, 6 and 8 (Fig. 3). The titer began to rise 8 to 12 days after the bacterial inoculation. The maximum titer ranged from 16 to 128.

We intended to induce abscesses only in the liver in the previous [2] and present studies. Extraphepatic abscesses were, however, found in some of cattle employed. To show the expected effect of extrahepatic abscess on levels of serum sialic acid and the others determined, we also induced abscess

Fig. 5. Effects, on serum sialic acid concentration, of sterile saline injection into portal vein (left arrows), bacterial inoculation into abdominal wall (center arrows) and surgical manipulation similar to that performed at center arrow with no bacterial inoculation (right arrows).

in the abdominal wall without affecting the liver.

A few drops of the bacterial suspension (Table 1) was inoculated on the cut surfaces of abdominal muscles in cattle Nos. 9 and 10. The inoculation was followed by pyrexia similar to or greater than that seen in the cases of hepatic abscess formation (Fig. 1) and by elevation of about 23 to 25 mg/dl in serum sialic acid level in both cases (Fig. 5). Antibody titer against *F. necrophorum* began to rise 11 to 19 days after the bacterial inoculation (Fig. 3). The maximum titers of cattle Nos. 9 and 10 were 32 and 16, respectively.

A large abscess was formed in each animal and debris was removed from a cavity about 8cm in diameter on the 8th (No. 9) and 14th day (No. 10) after inoculation. Its cavity was washed daily with acriflavin solution. The skin surface looked normal on the 29th day of postinoculation in both cases.

After the high levels of serum sialic acid returned to the preinoculation levels, both cattle were surgically treated to make cut surfaces similar to the previous ones in the abdominal wall of the opposite side. No bacterial suspension was inoculated. The treatments induced no change or slight elevation for a day in rectal temperature

Appendix 1. Correlation between serum sialic acid concentrations determined by enzyme-testing kit and the modified Ehrlich's method. $y=0.95x-5.6$, $r=0.90$ and $n=39$.

(Fig. 1). Levels of serum sialic acid were raised 10 to 15 mg/dl higher than those of the preinoculation (Fig. 5). It was intended not to make an abscess in the abdominal wall, but an abscess about 4 cm in diameter was found at autopsy in each case, although the skin surface looked normal.

DISCUSSION

Motoi *et al.* [3] and Motoi [6] examined several methods for measurement of blood sialic acid and obtained highly significant correlations among them. The Ehrlich's method was one of them. We could not, however, determine serum sialic acid concentration by the Ehrlich's method recommended by Motoi [6] due to precipitation of serum protein and to the large volume (0.5 ml) of serum to be analysed. The highly significant correlation they reported was not obtained in our hands. It was modified to avoid protein precipitation as described above. There was highly significant statistical correlation between the values obtained by the enzyme-testing kit and the modified Ehrlich's method (Appendix 1). Three per-

sons respectively obtained 1.5, 1.9 and 2.2% coefficient of variation in each 10 measurements by the modified method.

Sialic acid in the blood of cows with liver diseases has already examined in the U. S. S. R. [8], from which no detailed information was given. Motoi et al. [3] reported that the concentration of plasma sialic acid increased in cattle affected with hepatic abscess and that the increase was not specific to hepatic abscess. They also reported that levels of plasma sialic acid were in the normal range in some affected cattle with hepatic abscess which might not be at its acute phase. We support their field observations in the present study on experimental hepatic abscess.

Our evidence indicates that in experimentally infected animals serum sialic acid levels may be raised as a consequence of the formation of abscesses at a variety of sites in the body, and that it is not a response specifically to hepatic abscess. The experimental animals employed in the studies of Motoi and colleagues [4, 5] and affected with hepatic abscesses were also affected with extrahepatic abscesses, some of which were recorded in other collaborators' reports on the same animals [1, 7, 10]. These abscesses were found in the abdominal wall, lung and so on. Unfortunately the presence of these extrahepatic abscesses was not recorded in the former reports [4, 5] and so their possible influence was not taken into account.

The abscesses induced by the inoculation of *F. necrophorum* on cut surfaces of the incised abdominal wall in the present study were similar to those seen there in Motoi's animals affected with the hepatic abscesses [5]. The pyrexia and high level of blood sialic acid seen in association with these extrahepatic abscesses indicated that their formation would be as stressful to the host as the hepatic abscess. The abdominal wall of Motoi's unaffected and control animals

looked similar to that of the animals that were not inoculated with *F. necrophorum* in the present study. Rises in rectal temperature and blood sialic acid level were different between these two groups (Figs. 1 and 5).

Some of the experimental animals employed in the present study were affected with extrahepatic pathological lesions (Table 1). These extrahepatic lesions could not be neglected in their relation to the elevation of blood sialic acid concentration, because the level of blood sialic acid increased significantly in inflammatory diseases in cattle [3] and it also increased in cattle Nos. 9 and 10 affected with abscesses in the abdominal wall.

Hepatic abscesses were found without extrahepatic abscesses in cattle Nos. 4, 7 and 8. Cattle Nos. 7 and 8 had been affected, however, with dermatitis for a short period after blood sialic acid concentration reached a peak. Elevation of blood sialic acid concentration to the peak was, therefore, considered to have been induced in association not with the dermatitis but with the hepatic abscess. But no quantitative measurement of abscess formation has been made to establish this relationship.

Whether the elevated level of blood sialic acid decreases or persists (Fig. 4b or 4e) may be determined not by the presence of hepatic abscess itself but by intensity and range of inflammation occurring in and around the abscess. About 20 ultrasonographic lesions small in size disappeared before autopsy in cattle No. 8, but the level of blood sialic acid did not decrease. Inflammation might have existed in an around the remaining lesions.

An effect of the dermatitis on the level of blood sialic acid was not strictly evaluated in the present study, but it might have a much weaker effect, if any, on the level than the hepatic abscess had. Cattle No. 8 was affected with the dermatitis for a period

from the 25th to 40th day of post-inoculation. The level of sialic acid did not fluctuate much during this period (Fig. 4e), from which it could be deduced that the 2nd elevation seen in the sialic acid level after the inoculation in cattle No. 7 (Fig. 4d) might not have been due to the dermatitis observed but rather to the enlargement of the hepatic abscess detected in this animal [2] (Table 1).

Blood sialic acid level did not increase even 10 days after the bacterial inoculation in cattle No. 2, of which liver had multiple necrotic foci (Fig. 2). The mechanism responsible for the elevation of blood sialic acid level might be completely destroyed in such a severe case.

Both Takeuchi *et al.* [10] and Motoi *et al.* [4] recorded, in their reports on different facets of the same experiment, the antibody responses of cattle inoculated with *F. necrophorum*. Careful examination on these two papers may disclose 4 experimental animals they employed in common and some discrepancies between the reports. The three of them were affected with lung abscesses [10] and the antibody titer began to rise either 10 to 13 days [10] or 1 to 13 days [4] after the bacterial inoculation. The titer began to rise 7 to 11 days after inoculation in the present study. We could not confirm antibody responses with respect to hepatic abscess in the present study, because only one animal was subjected to the antibody examination among 3 cattle affected only with hepatic abscess. At least, we agree with Takeuchi *et al.* [10] in concluding that the determination of the antibody may be useful for the diagnosis of "*F. necrophorum* infection" in cattle.

Specificities and limitations of diagnostic methods being well accepted, a practical system of diagnosis of the bovine hepatic abscess needs to be developed. The simple and easy determination of blood acute phase reactants such as sialic acid or

mucoprotein [3] may be useful as a screening test for affected beef cattle in a herd. A specific but costly enzyme kit is not always needed for determination of sialic acid. Ultrasonographic diagnosis [2] can be applied on the selected animals. We hope that ultrasonographic information helpful in the clinical diagnosis of bovine hepatic abscess will soon become available.

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要 約

Fusobacterium necrophorum 接種牛における血清シアル酸：板底外茂雄・田宮和枝¹⁾・山本理恵子・佐藤真澄（農林水産省家畜衛生試験場，¹⁾埼玉県大宮家畜保健衛生所）——*Fusobacterium necrophorum* の培養菌液を7頭の牛の肝内門脈に接種したところ2頭は接種後10日と14日で死亡し，肝には壊死巣が密発していた。接種後12～126日に剖検した他の5頭の肝には膿瘍がみられ，一部は肺や肋間などにも膿瘍がみられた。肝に壊死巣の密発していた例のうち1頭では，接種後10日でも血清シアル酸の上昇はなかったが，他の1頭では8日に上昇していた。肝膿瘍がみられた例の血清シアル酸値は，接種後3～5日から上昇し，7～9日でピークに達し，その高値を2～3日間保ったのち，しだいに接種前値にもどる例もあったが4か月以上高値を持続する例もあった。さらに腹壁膿瘍を作出して，肝内あるいは肝外の膿瘍と血清シアル酸値とを比較検討し，肝膿瘍形成と血清シアル酸値上昇との関連性を明らかにした。