Pathology of Bovine Abortion and Newborn Calf Death Caused by Dual Infection with Chlamydia psittaci and Infectious Bovine Rhinotracheitis Virus

Hiroaki OGINO, Kaneyoshi KANEKO, Dai NAKABAYASHI, Taisei WATANABE, and Jinichi MURAYAMA

Kaetsu Livestock Hygiene Service Center, Toshin-chyo, Shibata 957, and *Chuo Livestock Hygiene Service Center, Nishikanbara, Niigata 959-04, Japan

(Received 14 March 1995/Accepted 20 September 1995)

**ABSTRACT.** Nine aborted fetuses and one newborn calf, diagnosed as Chlamydia psittaci (C. psittaci) infection, were pathologically examined. The characteristic lesions in the liver were focal necrosis in 9 aborted fetuses and granulomatous necrotic foci in the newborn calf. Moderate numbers of intranuclear inclusion bodies were found in necrotic foci of the liver, spleen, thymus, lymph nodes, adrenal gland, kidney, lung and forestomach. Immunohistochemically, a small number of C. psittaci antigens was demonstrated in necrotic foci of the liver and correlated with distribution of elementary bodies. Moderate numbers of infectious bovine rhinotracheitis (IBR) virus antigens were also detected in degenerating and necrotizing parenchymal cells in various organs and correlated with distribution of intranuclear inclusion bodies. Thus, these aborted bovine fetuses and newborn calf were interpreted as being dually infected with C. psittaci and IBR virus during pregnancy. — **KEY WORDS:** aborted fetus, Chlamydia psittaci, IBR virus.

---

**RESULTS**

Pathology: Gelatinous degeneration was observed in the subcutaneous tissues of aborted animals. The lesions were especially prominent in the mandibular and umbilical areas. In 2 cases (Nos. 4 and 5), a few white focal lesions (about 2 to 5 mm in diameter) were scattered in the skin. The thoracic fluid and abdominal fluid were brownish-red in color. The livers were softened, enlarged, and discolored yellowish-red in all the aborted cases. Nine fetuses revealed small necrotic foci in the liver, and seven showed the enlarged lymph nodes. Autolysis of the brain was pronounced and only in 3 aborted cases (Nos. 2, 3, and 8) it could be examined. In other respects, the fetuses appeared grossly normal. In the newborn calf, the liver with small-sized necrotic foci was slightly enlarged. Hemorrhagic changes were observed in the alimentary tract, thymus, kidney and adrenal gland.

Microscopical findings were characterized by focal necrosis and hemorrhages in the various organs such as the liver, spleen, lung, thymus, adrenal gland, lymph nodes and central nervous system (CNS). The distribution of necrotic lesions is shown in Table 1.

Necrotic foci in the liver were more numerous in the centrlobular midzonal areas (Fig. 1). Many lobules contained more than one focus of necrosis, without cellular reaction. Small-sized granulomatous necrotic foci were observed in the liver of a newborn calf (Fig. 2). Necrotic foci with karyorrhexis of lymphocytes and reticular cells were found in the spleen, thymus and lymph nodes. The cortex of thymus was more severely affected than the medulla. In these tissues, hemorrhagic lesions were more remarkable than other tissues examined. The necrotic lesions were also recognized in the adrenal cortex and dermis. In the CNS, neuronal degeneration and necrosis

---

**Chlamydia psittaci (C. psittaci)** has been recognized as one of causative agents of abortion in cows [1, 3, 5, 8, 15]. Recently, an outbreak of bovine abortion occurring in Niigata Prefecture has been reported to be caused by C. psittaci [10]. In an attempt to extend these observations, we reexamined the same cases using the pathological and immunohistochemical methods. As a result, it was clearly demonstrated that most of the cases were dually infected with C. psittaci and infectious bovine rhinotracheitis (IBR) virus. The present paper describes the results obtained in these observations.

**MATERIALS AND METHODS**

Sixteen aborted fetuses and one calf that died at 3 days of age were necropsied. Seven aborted animals were in advanced stages of autolysis and could not be examined. Tissues of the nine aborted cases and one newborn animal were fixed in 10% neutral buffered formalin, embedded in paraffin, sectioned and stained with hematoxylin and eosin (HE) and Gimenez modification of Macchiavello staining.

Immunohistologically, C. psittaci and IBR virus antigens and immunoglobulin (IgG and IgM)-containing cells were demonstrated by the avidin-biotin-complex (ABC) immunoperoxidase method, as described previously [12]. Anti-C. psittaci rabbit serum provided by Dr. K. Hirai, Gifu University, Gifu, Japan, and Anti-IBR virus rabbit serum supplied by Dr. S. Taniguchi, Kyoritsu Shoji Co., Ltd., Central Research Laboratory, Ibaraki, Japan were used at a dilution of 1:4,092 and 1:256, respectively. Anti-bovine IgG (Fc) rabbit serum (EY Lab) and anti-bovine IgM (u) rabbit serum (Miles Labo) were used at a dilution of 1:1,000 and 1:2,000, respectively. C. psittaci antigen was demonstrated by using an autoclaving method [4]. Serum from a non-immunized rabbit was used for the control study.
were observed in the medulla oblongata of 3 fetuses (Nos. 2, 3, and 8). Intranuclear inclusion bodies associated with the necrotic foci were found in reticular cells of the spleen, lymph nodes and thymus, parenchymal cells of the adrenal gland (Fig. 3), and bronchial epithelial cells of the lung. In addition to these tissues, the calf had intranuclear inclusion bodies in the epithelial cells of the forestomach and collecting tubules of the kidney.

Chlamydial elementary bodies stained by the Gimenez method were detected in degenerative cells of the livers of 7 aborted fetuses (Fig. 1) and a calf (No. 10), the lymph nodes of a fetus (No. 7) and the skin of a fetus (No. 5). Their distribution corresponded with that of focal necrosis.

**ImmunohistoLOGY:** The distributions of *C. psittaci* and IBR virus antigens are shown in Table 1. In the aborted fetuses, *C. psittaci* antigen was detected in the cytoplasm (Fig. 4) and IBR virus antigen appeared in the nucleus and cytoplasm of degenerating and necrotizing parenchymal cells (Fig. 5). The distribution of *C. psittaci* antigen was correlated with that of elementary bodies. The correlation was also observed between distributions of IBR virus antigen and intranuclear inclusion bodies in necrotic foci. Moreover, both antigens were detected in the same necrotic foci in the liver. In the dead newborn calf, *C. psittaci* antigen was found only in granulomatous necrotic foci of the liver (Fig. 6). On the other hand, IBR virus antigens were more widely distributed in neuronal cells in the CNS, parenchymal cells of the adrenal gland, tubular epithelial cells of the kidney, alveolar cells of the lung and epithelial cells of the forestomach. No IgG- and IgM-containing cells were detected in peripheral necrotic areas of the spleen and lymph nodes of the aborted fetuses.

**DISCUSSION**

In a previous study [10], *C. psittaci* was isolated from the livers of aborted fetuses and a dead calf. In the present study, characteristic lesions in the livers were focal necrosis in the aborted fetuses and granulomatous necrotic foci in a newborn calf. A small number of elementary bodies were detected in focal necrosis and granulomatous necrotic foci of the liver. Moreover, moderate numbers of intranuclear inclusion bodies were also found in the necrotic foci of the liver, spleen, thymus, lymph nodes, adrenal gland, kidney and forstomach. The focal necrosis accompanying with intranuclear inclusion bodies bore a close resemblance to that in the aborted fetuses induced naturally or experimentally by IBR virus infection [6, 7, 9, 14, 17], except the presence of elementary bodies characteristic of *Chlamydia*.

Recently, techniques for detecting infectious agents in fixed, paraffin-embedded tissues have been much improved [4, 11, 12]. In the present cases, *C. psittaci* antigens were demonstrated in necrotic foci of the liver by the use of the autoclaving method [4], and well correlated with the presence of elementary bodies. Moderate amounts of IBR virus antigens were also detected in degenerating and necrotizing reticular cells and parenchymal cells, and distribution of antigens correlated with that of intranuclear inclusion bodies. Moreover, both antigens were located in the same necrotic foci of the liver. Thus, our results evidently indicated that the present cases were caused by dual infection with *C. psittaci* and IBR virus.

The lymphoid organs especially in the spleen and lymph nodes in bovine fetuses over 5th month of gestation are activated and IgG levels are elevated [2, 11–13]. The calf from a heifer inoculated with bovine viral diarrhea-mucosal disease (BVD-MD) virus at 116 days of gestation had presuckling serum neutralizing titer against BVD-MD virus [16]. In the present cases, fetuses ranging in age from 129 to 219 had no IgG- and/or IgM-containing cells in peripheral necrotic areas of the lymphoid tissues. The absence of

---

**Table 1. Distribution of necrotic lesions, *C. psittaci* and IBR virus antigens in aborted fetuses and newborn calf**

<table>
<thead>
<tr>
<th>Animal number</th>
<th>Liver</th>
<th>Spleen</th>
<th>Lung</th>
<th>Thymus</th>
<th>Lymph node</th>
<th>Skin</th>
<th>Adrenal gland</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>+/-1/0</td>
<td>0/0</td>
<td>0/0</td>
<td>0/0</td>
<td>0/0</td>
<td>N.D</td>
<td>0/0</td>
</tr>
<tr>
<td>2</td>
<td>+/-1/1</td>
<td>0/0</td>
<td>0/0</td>
<td>0/0</td>
<td>0/0</td>
<td>N.D</td>
<td>0/0</td>
</tr>
<tr>
<td>3</td>
<td>*/+/3/3</td>
<td>*/+/2/0</td>
<td>0/0</td>
<td>*/+/2/0</td>
<td>*/+/3/0</td>
<td>N.D</td>
<td>*/+/3/0</td>
</tr>
<tr>
<td>4</td>
<td>*/+/3/2</td>
<td>*/+/3/0</td>
<td>*/+/3/0</td>
<td>*/+/2/0</td>
<td>*/+/3/0</td>
<td>N.D</td>
<td>*/+/3/0</td>
</tr>
<tr>
<td>5</td>
<td>*/+/2/3</td>
<td>*/+/3/0</td>
<td>*/+/2/0</td>
<td>*/+/2/0</td>
<td>*/+/3/0</td>
<td>N.D</td>
<td>*/+/3/0</td>
</tr>
<tr>
<td>6</td>
<td>*/+/0/0</td>
<td>*/+/0/0</td>
<td>*/+/0/0</td>
<td>*/+/0/0</td>
<td>*/+/0/0</td>
<td>N.D</td>
<td>*/+/0/0</td>
</tr>
<tr>
<td>7</td>
<td>*/+/3/3</td>
<td>*/+/2/0</td>
<td>*/+/2/0</td>
<td>*/+/2/0</td>
<td>*/+/3/0</td>
<td>N.D</td>
<td>*/+/3/0</td>
</tr>
<tr>
<td>8</td>
<td>*/+/3/3</td>
<td>*/+/1/0</td>
<td>*/+/1/0</td>
<td>*/+/1/0</td>
<td>*/+/0/0</td>
<td>N.D</td>
<td>*/+/2/0</td>
</tr>
<tr>
<td>9</td>
<td>*/+/3/3</td>
<td>*/+/1/0</td>
<td>*/+/1/0</td>
<td>*/+/1/0</td>
<td>*/+/0/0</td>
<td>N.D</td>
<td>*/+/2/0</td>
</tr>
<tr>
<td>10</td>
<td>*/+/0/2</td>
<td>*/+/0/0</td>
<td>*/+/1/0</td>
<td>*/+/0/0</td>
<td>*/+/0/0</td>
<td>N.D</td>
<td>*/+/3/0</td>
</tr>
</tbody>
</table>

*: Dead newborn calf.

Necrotic foci/IBR virus antigen by ABC/Chlamydia psittaci antigen by ABC.

Degree of lesion: -, negative; +, slight; ++, moderate; ++++, severe.

Positive number of antigens = 0, negative; 1, small; 2, moderate; 3, large.

○: Intranuclear inclusion body.
DETECTION OF C. PSITTACI AND IBR VIRUS

Fig. 1. (a) Focal necrosis and (b) Chlamydial elementary bodies (arrows) in the liver of an aborted fetus. a, HE stain, × 100 and b, Gimenez method, × 750.

Fig. 2. Small-sized granulomatous focus in the liver of a newborn calf. HE stain, × 400.

Fig. 3. Intranuclear inclusion bodies in parenchymal cells of the adrenal gland of an aborted fetus. HE stain, × 1,000.

Fig. 4. C. psittaci antigen in degenerating and necrotic foci of the liver of an aborted fetus. Immunoperoxidase (IP) stain, × 200.

Fig. 5. IBR virus antigen in the nucleus and cytoplasm of necrotizing liver parenchymal cells of an aborted fetus. IP stain, × 100.

Fig. 6. C. psittaci antigen in granulomatous necrotic foci in the liver of a dead calf. IP stain, × 400.
immunoglobulin-containing cells and no reactivation of the lymphoreticular system in the aborted fetuses were considered may be due to rapid death after infection with IBR virus and/or C. psittaci.

ACKNOWLEDGMENTS. The authors thank to Mr. Ando and T. Fujisawa, National Institute of Animal Health, for preparing the photographs.

REFERENCES