Mem. Fac. Agr. Kagoshima Univ., **21**, 167–174, 1985

Acute Leptospirosis in a Calf — Possibly Preceding Seroconversion of the Herd

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Received for Publication September 10, 1984

Introduction

Systematic studies of bovine leptospirosis in Japan were first undertaken on the regional outbreaks in the northern part of Hyogo Prefecture in 1949–1950, in which infection of *L. autumnalis*, *L. australis* and *L. hebdomadis* was serologically detected and *L. hebdomadis* was isolated[14]. The other two types were later isolated in this region[3]. This was the first evidence of bovine leptospirosis by these serotypes in the world[10]. The main signs of the disease were hemoglobinuria, anemia and anorexia, adding fever and icterus in severe cases[13]. Milking cows lowered their milk production and secreted bloody or thickened milk. Some died in acute phase and most of the cases recovered from the illness. Abortion associated with leptospiral infection was reported with special emphasis on asymptomatic reservoirs[11], though not so severe as in the cases of the United States where *L. pomona* was the prevailing serotype. Distribution of leptospirosis in cattle in western Japan has been immunologically proved[12]. In southern Kyushu, only an outbreak of hemoglobinuria in grazing cattle associated with high antibody titers against *L. hebdomadis* has been reported[7], while 4 representative serotypes have been proved in the sera of horses with "moon blindness"[10].

Recently, we had a chance to observe a calf showing hemoglobinuria, in whose kidney and liver were detected numerous leptospiral organisms by silver-impregnation. The calf had no antibody to pathogenic serotypes, but serological study 5 months after the outbreak proved seroconversion of the maternal herd to *L. autumnalis*. In the present paper we report the acute septicemic case of leptospirosis.

Materials and Methods

Animal: A 50-day-old female calf of Japanese Black breed, weighing 31.5 kg, born and bred in a certain pasture in Satsuma County, Kagoshima Prefecture.

History: In the morning of Oct. 3, 1983, the calf was found excreting hemoglobinuria in a state of depression and anorexia. Having been kept under observation, the calf died in the evening and the next morning was conveyed to our laboratory for pathological examination.

Microscopic studies: The calf was autopsied and the main organs were removed, fixed in 10% formol solution, embedded in paraffin, cut into sections 4–5 μm thick and were stianed with hematoxylin and eosin (HE) for microscopy. Special stains such as periodic acid-Schiff (PAS), azan (Mallory-Heidenhain) and Berliner blue staining for iron were carried out. Levaditi's

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silver impregnation method for spirochaeta was used for tissues of the liver and kidney.

Serological study: Five months after the outbreak, we visited the pasture to collect the blood of the herd to which the calf and her mother belonged. Unfortunately, the mother had already been eliminated, and we obtained sera from 8 adult cows in the same herd as hers, aging 3–14 (average 5.6) years. The state of antibody production against leptospira in both the calf and the maternal herd was examined by a modified agglutination test of Shüffner and Mohotar[6,8,9] using 5 representative serotypes, *L. autumnalis*, *L. hebdomadis*, *L. australis*, *L. canicola* and *L. icterohemorrhagiae* as antigens.

Results

Gross findings: Nutritional conditions were estimated to be within the normal range. The visible mucous membranes in the conjunctiva and mouth were pale or faintly jaundiced. Abdominal subcutaneous tissue and visceral adipose tissue took slightly more yellowish hue than usual. The liver, weighing 1,080 g, showed reddish brown color both on the capsular and cut surfaces with its consistency and blood content of normal ranges. The gall bladder was filled with yellowish brown, viscous or gelatinous bile. After formol-fixation the liver took a greenish tinge with small white spots scattered on the capsular surface. Kidneys looked dark purple in color with slight swelling and the capsules were easily stripped off, uncovering dense petechiae and many whitish spots on the cortices. Boundaries of the 3 layers on the cut surface were somewhat indistinct. The urinary bladder contained about 100 ml of hemoglobinuria. The contents in the digestive tract were scanty. The spleen presented a slight swelling and revealed distinct traveculae, obscure follicles and a small amount of blood, when made a cut. The lung was a little inflated with air, containing dark catarrhal areas in the anterior and intermediate lobes. Dense petechiae were observed on epicardium along with the left longitudinal sulcus and a part of coronary sulcus. The right ventricule contained a large amount of clot and in the left ventricule a small number of petechiae were found on the endocardium. Petechiae were seen on the serous surface of the cecum and in the thymus. Slight swelling of the mesenteric lymph nodes was recognized.

Histological findings: In the renal cortex, many small foci of hemorrhage were found in the subcapsular area. The other foci stained pale with HE were areas showing edema and moderate increase in amount of connective tissue. Epithelial degeneration, desquamation and regeneration were occasionally encountered in the proximal tubules and some tubules were precipitated with brown pigments. Berliner blue reaction was a little stronger in the distal tubules than in the proximal ones which showed a weak positivity in general, distributed diffusely in the cytoplasm. Some brown pigments remained negative in the proximal tubules. Mononuclear round cell infiltration was observed around small arteries and glomeruli, whereas no remarkable change was seen in the glomeruli, except a few showing reduction in size in the fibrous-edematous area (Fig. 1). Urinary casts were seldom seen and no particular changes were recognized in the medulla.

Periportal infiltration of small round mononuclear cells and derangement of hepatic cell cords were evident in the liver, the latter occasinally showing a tendency to dissociation of the liver cells (Fig. 2). Slight degenerative changes of hepatocytes and bile thrombi in the bilary canaliculi were obviously seen in the centrilobular or paracentral area (Fig. 3). Numerous Kupffer cells and mononuclear phagocytes were found containing brown pigments on the sinusoidal wall as well as in the lumen. In the central or paracentral zone there often were focal vacuolated areas composed of hepatocytes each having an intracytoplasmic inclusion with a distinct halo (Fig. 3). The sizes of

inclusion bodies were various but smaller than the erythrocytes with stainability from yellowish-pink to reddish-orange. Sometimes two or more inclusions were seen in a cell. The Kupffer cells and phagocytes in the sinusoids revealed a marked positive reaction to the Berliner blue staining (Fig. 4). Even in the central and portal veins were found many iron-bearing cells. Erythrophagic cells were occasionally encountered.

The spleen showed remarkable congestion, atrophy of lymph follicles and erythrophagia, while hemosiderosis was not so conspicuous. In the lung were found various pneumonic changes, such as exudation of serum, fibrin, red blood cells and neutrophils in the alveoli, edematous dilatation of lamina propria of bronchioles with denudation of the mucous epithelia and distention of interlobular septa with stagnation of lymph. Bacterial colonies were seen in the alveolus sometimes. The tonsils contained larger colonies of bacteria. The lymph nodes presented lysis of the secondary nodules and diffuse proliferation of macrophages and lymphocytes. Hemorrhage in the thymus was located largely in the medulla. Minute extravasation was noticed in the molecular layer of the cerebellum. Despite of careful examinations, none of the piroplasma-like organism was discovered in the red blood cells in the tissue section.

By the silver-impregnation method a number of slender, spiral organisms with hooked ends, indistinguishable from those of leptospira, were disclosed in the liver and kidney (Figs. 5, 6, 7 and 8). Most of them were seen singly, sometimes 2 or 3 held together, in and around the renal intertubular capillaries and hepatic sinusoids. A fairly large number of organisms were found in the wall of renal small arteries and hepatic portal veins. Some organisms appeared to be parasitized in the lumen or epithelium of the renal tubules. The great majority of the organisms were detected in the cortex, very few in the outer medulla and almost none in the inner medulla. No special correlation was observed between cell infiltration and localization of the organisms.

Serological study: The results of antibody detection using 5 pathogenic serotypes of *Leptospira interrogans* are shown in Table 1. All 8 cows of the maternal herd turned out to be positive to *L. autumnalis*, ranging from + to ++, while the calf was negative to all the serotypes examined. There was an apparent tendency that the older ones possessed the higher titers. As an exceptional case, the oldest 14-year-old cow having the highest titer to *L. autumnalis* proved to be sensitized by *L. icterohemorrhagiae*, too. This animal died within 10 days after our sampling of the blood for the present study.

Table 1. Agglutinating titers of the infected calf and her maternal herd to pathogenic serotypes of leptospira five months after the outbreak

Animal No.	1	2	3	4	5	6	7	8	The Present Case
Sex	F	F	F	F	F	F	F	F	F
Age	4Y	4Y	10Y	3Y	3Y	3Y	14Y	4Y	50D
Leptospira autumnalis	++	+	++	+	+	+	+++	+	−
Leptospira hebdomadis	−	−	−	−	−	−	−	−	−
Leptospira australis	−	−	−	−	−	−	−	−	−
Leptospira canicola	−	−	−	−	−	−	−	−	−
Leptospira icterohemorrhagiae	−	−	−	−	−	−	+	−	−

Key; $+ < 10^2$, $++ < 10^3$, $+++ > 10^6$ Y: Years Old, D: Days Old, F: Female

Discussion

A calf excreting hemoglobinuria died in a pasture of Satsuma County, Kagoshima Prefecture and it was proved to have many characteristics of leptospirosis by macro- and microscopic examinations, including detection of the leptospiral organisms by Levaditi's method. Though the calf had no antibodies to pathogenic serotypes of leptospira, the herd to which the calf and her mother belonged showed seroconversion when examined 5 months after the outbreak.

As the present case was so young a calf that we had to choose data on calves of the nearest age for comparison, namely those of the experimental and the naturally occurring cases in the United States[1,2]. Although febrile stage of the present case was overlooked, fever lasting 1–3 days coincident with spirochetemia was the main clinical sign of the experimentally infected calves, occurring 2–7 days after inoculation[1]. Slight anorexia was noted during the febrile period and after termination of fever all appeared normal except those dying in 1–2 days, in which hemoglobinuria signaled the approach of death. Our calf might have been found in such a moribund stage probably showing subnormal temperature. Epicardial petechiae were common in the naturally infected cases as well as alveolar edema with occasional exudation of fibrin and leukocytes in the lung, generally juicy lymph nodes and small hemorrhage in the thymus[2]. Numerous petechiae on the cortical surface under stripped renal capsule in the natural cases well corresponded with those of the present case. Widespread foci of mononuclears found in the experimental cases were comparable to the foci of mild fibrosis and edema infiltrated with mononuclear cells in the renal cortex of our calf. Generally speaking, the present calf exhibited much milder changes in nephrosis and interstitial nephritis than those of the naturally or the experimentally infected calves. Alterations in the liver of the calf were also milder than those in the naturally occurred cases. The calf manifested no definite central necrosis, not showing lipidosis, cloudy swelling nor hyaline droplet degeneration, but only subtle degenerative changes assoicated with bile stasis in the centrilobular and paracentral areas, which was in accord with toxic jaundice in leptospirosis[5]. Portal lymphocytic infiltration and erythrophagia in the spleen, together with bile stasis, were the principal points of agreement with the natural cases. In our case, hemosiderosis in the Kupffer cells and renal tubular epithelia as well as erythrophagia suggested hemolytic anemia, which could be attributed to leptospira[5], as no obvious organism of piroplasma was detected in the red blood cells. One point of difference was the intracytoplasmic inclusions in the liver cells of the calf. At a glance they might be confused with focal vacuolar degeneration or focal fatty areas. The exact nature of them was to be clarified, though we presumed them as non-specific tissue reaction.

The organisms stained by Levaditi's method in the kidney and liver were indistinguishable from those of the leptospira, showing slender, elongated, sharply delineated, black staining bodies with hooked ends. In the present case the organisms were so numerous in the kidney and were essentially confined to the cortex. Most of them were found in association with vasculature and far less in the tubular lumen, which differed from the natural cases in the United States. As tissue damage was very mild in the calf, no particular correlation was observed between lesions and the numbers of organisms. Not only the hepatic sinusoids but also the wall of portal veins showed a high affinity to the organisms. Diffuse distribution of the organisms associated with the vascular system may indicate a septicemic state without participation of the antibody.

Prevalence of one serotype, *L. autumnalis*, in the maternal herd of the calf was demonstrated by serological study performed 5 months after the outbreak, which signified that there was a chance of exposure to the serotype in the past. The calf turned out to be negative to all the serotypes examined.

IgM antibody production associated with the agglutination test would decrease after several weeks of primary response and if the mother cow had had the antibody, the calf could have retained agglutinating titer by this age[4]. To our regret we could not examine the mother, for she had already been eliminated. We are prone to suppose that the mother had no acquired immunity to the leptospira and that the herd to which she belonged was exposed simultaneously with the outbreak. This conjecture, based upon a speculation that the calf was affected by *L. autumnalis*, needs corroborative evidence using immuno-histological techniques.

Further study will be required for isolation of the agent with continual investigations.

Summary

In Oct. 21, 1983, a 50-day-old female calf in Satsuma County, Kagoshima Prefecture, died shortly after showing signs of depression and hemoglobinuria and was submitted to pathological examination. Macro- and micro-scopic findings suggested acute leptospirosis a little milder than usual: hemolytic anemia, epicardial petechiae, diffuse petechiae and pin-point sized white spots on the surface of the kidney, mild nephrosis and interstitial nephritis, portal lymphocytic infiltration, derangement of hepatic cell cords and centrilobular degenerative changes associated with bile thrombi. Tissues from the liver and kidney stained with Levaditi's method revealed numerous organisms indistinguishable from those of leptospira and most of them were found in or about blood vessels. Serological study, undertaken 5 months after the outbreak, showed a high prevalence of a serotype, *L. autumnalis*, in the maternal herd, while the calf was proved to be negative. These results implied that the calf died of acute septicemic leptospirosis lacking the protection of antibody and that the maternal herd might have been sensitized coincidently with her fatal infection.

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Explanation of figures

Fig. 1. Photomicrograph of the renal cortex in the calf, showing mononuclear cell infiltration around the glomerulus and small artery as well as interstitial edema and fibrosis surrounding dilated tubules lined with regenerated epithelia. Azan staining. ×150
Fig. 2. Portal mononuclear cell infiltration and derangement of the hepatic cell cords showing a tendency of dissociation of the liver cell. Hematoxylin and eosin (HE). ×150
Fig. 3. High power view of the liver section focussed on paracentral area with the central vein upper left. Note bile thrombi in the biliary canaliculi and intracytoplasmic inclusions each surrounded by a halo. HE. ×450
Fig. 4. High magnification of the liver section. Kupffer cells and phagocytes in the sinusoids showing positive reaction to iron stain. Berliner blue staining. ×450
Fig. 5. Silver-impregnated renal cortex showing a slender, elongated, spiral organism with hooked ends, indistinguishable from leptospira. Levaditi's staining. ×1000
Fig. 6. The same as Fig. 5. ×1000
Fig. 7. Silver-impregnated liver section showing a spiral-shaped organism. Levaditi's staining. ×1000
Fig. 8. Silver-stained renal cortex showing two leptospira organisms on the brush border of the tubular epithelia. Levaditi's staining. ×600


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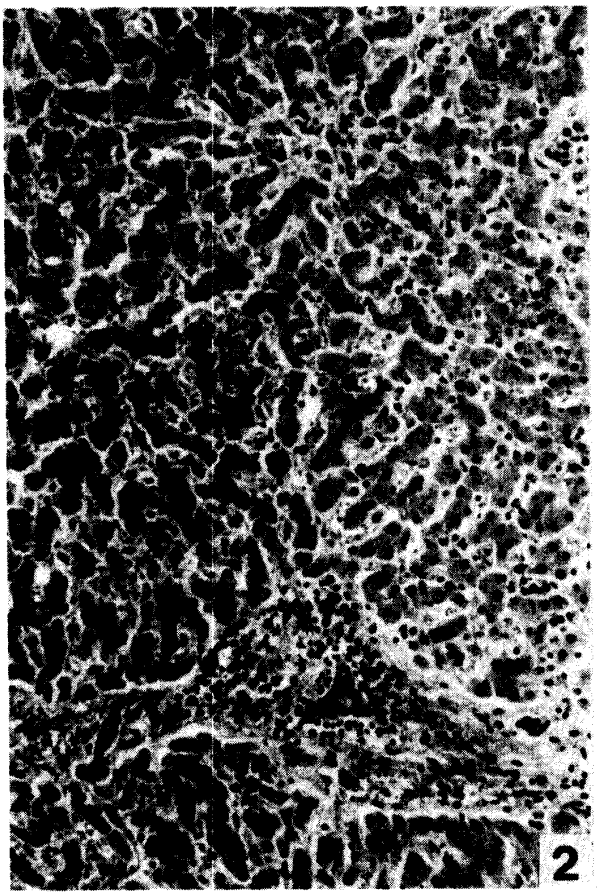
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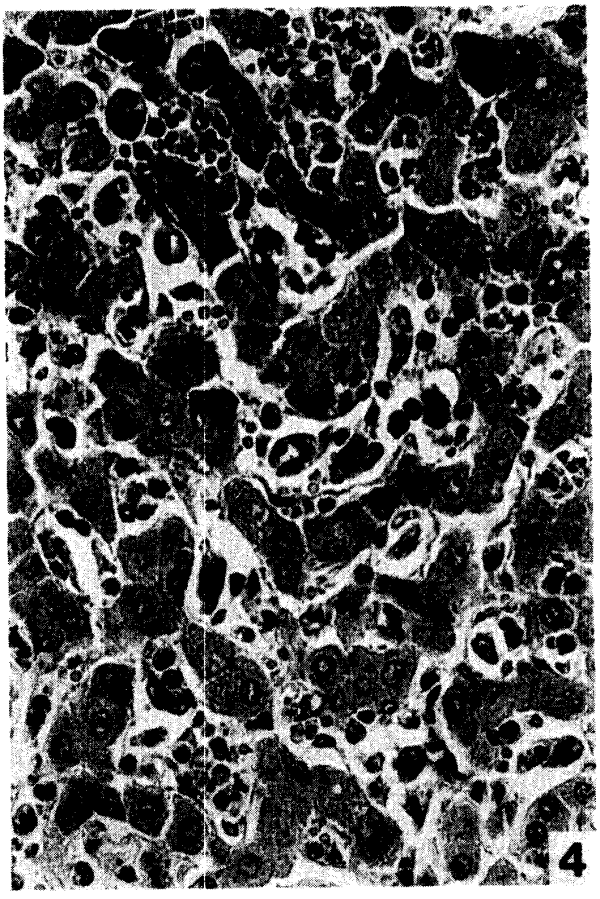
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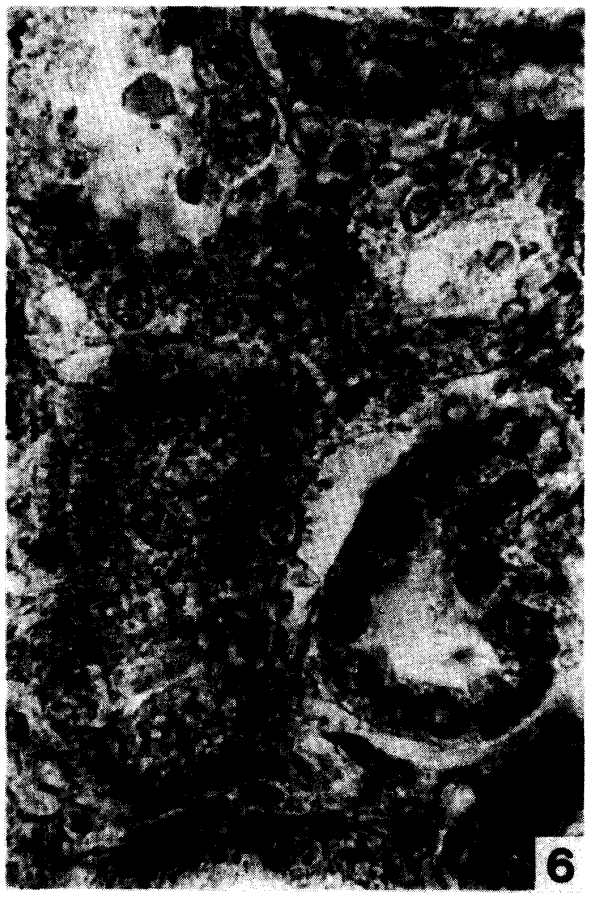

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