ENDOTOXIN-INDUCED GANGRENOUS MASTITIS IN COWS

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The scaling up of daily cow husbandry in recent years has naturally demanded a labor saving in its management and abuses of antibiotics have led to the increased incidence of microbial flora substitution in animal. Under these circumstances, gangrenous mastitis in dairy cows has been found to occur more frequently in many areas. This desease is characterized by a sub-acute course with both systemic symptoms and local symptoms of the udder resulting in the disuse or death of affected animals. Thus,this disease is now considered to be a serious problem for livestock hygiene.

At present, however, many aspects concerning the actual conditions and the developmental mechanisms of this disease are obscure, and few systematic studies have reported on the detection of the bacteria or the hemato-biochemical findings. Accordingly, there is still considerable argument of this subject. On the basis of only the clinical symptoms and the hematological or histopathological findings, it is assumed that gangrenous mastitis may be related to intoxication induced by endotoxin of Gram-negative bacilli.

The author preformed a fundamental study of endotoxin in livestock, while attempting to detect endotoxin in gangrenous mastitis cases occurring in the field. It was found that, in most cases, the disease was induced by intoxication associated with endotoxin of Gram-negative bacilli. In addition, clinical,

bacteriological, pathological and hemato-biochemical examinations were carried out for the purpose of investigating the developmental mechanisms of the disease.

The results are summarized below.

1. Incidence

The incidence of gangrenous mastitis was investigated for 11 years between 1974 and 1984 in Awaji, Hyogo prefecture. The total incidence was 794, which corresponded to 4.9% of 16,142 deaths in total and 38.4% of 2,059 deaths due to mastitis. There was a yearly increase in numbers.

The time of incidence was closely related to calving. Of 251 cases, 204 (81.3%) showed the symptoms within 7 days after calving and 197 out of 251 cases (78.5%) died or were put out of use within 3 days after manifestation of the symptoms due to the rapid course of the disease.

The main symptoms included systemic symptoms such as lethargy, anorexia, cardiopalmus, astasia, and diarrhea with malodor, and local symptoms of the udder such as purpura, cold sensations, swelling of the udder and changes in milk quality.

2. Bacteriological Investigation

Bacteria, pathology, blood and endotoxin were investigated for 11 years between 1974 and 1984 in 65 Holstein cows clinically diagnosed with gangrenous mastitis, 12 cows with acute mastitis, and 26 healthy cows as controls.

Bacteriological examinations of milk of the cows with gangrenous mastitis revealed the following Gram-negative bacilli : Escherichia coli in 33 out of 65 cases (50.8%) ; Pseudomonas aeruginosa in 11 out of 65 (16.9%) ; Klebsiella pneumoniae in 3 out of 65 (4.6%) ; Enterobacter aerogenes in 3 out of 65 (4.6%) ; Alcaligenes faecalis in 1 out of 65 (1.5%) ; and Acinetobacter anitratus in 1 out of 65 (1.5%). This indicates that Gram-negative bacilli accounted for 80.0% of all cases. The following Gram-positive bacteria accounted for 20.0% (13/65) : Bacillus cereus ; Clostridium perfringens ; Streptococcus sp. ; Actinomyces poygenes ; and Staphylococcus aureus.

In cultures of bacteria obtained from the main organs, the etiological bacteria were isolated in an almost pure state from the affected udder, but could be hardly isolated from the liver, the spleen or the kidney. From the results, it was concluded that the disease is caused by the action of endotoxins of the etiotogical bacteria.

According to the O serotype classification, 24 strains of isolated E. coli were categorized as follows : 5 strains in O2 ; 2 each in O8, O78 and O88 ; 1 each in O9, O64, O76 and O89 ; and 9 strains could not be evaluated. On the other hand, the serotype of 22 strains of P. aeruginosa was classified as follows : 6 strains in G ; 3 each in E, F and I ; 2 in A ; and 1 each in B and K. Since both species showed rather limited serotypes in this manner, it was

considered that these bacteria, derived from feces or the intestinal tract, are activated by intrinsic or extrinsic sensitization to induce the disease.

3. Histopathological Investigation

Pathologic autopsy revealed marked edema and petechial hemorrhage in the subcutaneous tissue by decorticating the affected udder. The incised surface had a dark red or light-brown color with a severe hemorrhage and edema of the interstitial tissues over an extended area, and the lesion was clearly separate from healthy areas.

The findings of the main organs were as follows : the liver was slightly swollen and showed pimelosis of a light yellow color and congestion ; hyperemia and partial petechia were observed in the kidney ; the spleen was swollen showing an unclear substrate ; and dark red spots were recognized in the lung and the heart.

Histopathological findings revealed hemorrhages inside the glandular alveoli of the udder, degenerative necrosis of epithelial cells of the alveoli, and edema and fibrinous necrosis of the blood vessel. Thrombus formation was observed in the affected udder, the liver, the kidney and the lung, in this order of frequency, which is a very similar finding to that of intravascular coagulation syndrome of endotoxemia in man. Lesions of the main organs were as follows : fatty degeneration hemorrhage, necrosis and thrombus formation in the

liver ; degenerative necrosis of epithelial cells of the uriniferous tubule in the kidney ; congestion and hemorrhage of the lung ; and small necrotic lesions of the heart. Thus, it was considered that endotoxin directly causes tissue damage, leading to severe symptoms.

4. Hemato-biochemical Investigation

The cows with gangrenous mastitis showed increases in red blood cells, hematocrit and stab leucocytes, and decreases in white blood cells, eosinophils, filamented neutrophils and monocytes. A decrease in filamented neutrophils, in particular, is concidered to be due to their transfer in a large amount into the affected udder and the margination of neutrophils stimulated by endotoxin.

There were decreases in total serum protein, albumin, globulin and calcium, and increases in serum urea nitrogen and inorganic phosphorus. Concerning serum oxygen, values of GOT, GPT and LDH were significantly high, while those of ALP and γ-GTP were rather high in general. In contrast, the activity of these enzymes was considerably decreased in the affected mammary gland tissues and the main organs. These enzymes may have been released into the blood following endotoxin-induced damage of the organs, particularly destruction of the mammary gland tissues.

In tests of the blood coagulation system, the following results were obtained : a decrease in platelet count ; prolongation of prothrombin time and

partial active thromboplastin time ; and a marked decrease in plasma fibrinogen. These results are very similar to the findings of intravascular coagulation syndrome of endotoxemia in man.

5. Investigation of Endotoxin

A fundamental study of endotoxin was carried out in order to clarify developmental mechanisms and prognosis of gangreous mastitis in cows. The finding that plasma and milk of healthy cows have nonspecific amidase activity, which directly hydrolyzes synthetic substrates, allowed the author to establish a new, highly precise and reproducible technique for measuring endotoxin in plasma and milk. This technique employs a synthetic coloring substrate method, following the removal of factors affecting limulus reaction by pretreatment with PCA.

Using the new technique, the quantity of endotoxin was measured in healthy cows (n=36), all of which showed 10 pg ml^{-1} or less. In the cows with acute mastitis from which Gram-negative bacilli were separated (n=12), endotoxin in plasma was always detected at 10 pg ml^{-1} or less, but it was found in milk at high concentrations of 1,147.0 ± 766.8 pg ml^{-1}. However, in all cases from which Gram-positive bacteria were separated (n=6), endotoxin concentrations in both plasma and milk were 10 pg ml^{-1} or less.

Among the cows with gangrenous mastitis in the Gram-negative bacterial infection group (n=30), endotoxin concentrations in plasma showed a high value of 89.6 ± 68.2 pg ml^{-1}, while those in milk reached a very high value of 9.1 ± 5.2 × 10^6 pg ml^{-1}. This stongly suggested that the disease induced by Gram-negative bacterial infection is associated with endotoxemia. In the Gram-positive bacterial infection group (n=3), endotoxin in plasma and milk was always detected at 10 pg ml^{-1} or less.

The endotoxin positive cases with gangrenous mastitis tended to show severe clinical symptoms and a short period before disuse, compared with the negative cases. In addition, endotoxin was detected with high specificity by the endospecie test, in which only those parts of the path activated by endotoxin were reconstructed and a synthetic coloring substrate was added. This finding thus confirmed that endotoxin was a developing factor of the disease in the Gram- negative bacterial infection group.

Since endotoxin was detected at high concentrations in plasma and milk of the Gram-negative bacterial infection group by the limulus test using the synthetic coloring substrate method, endotoxin may be considered to be a useful marker for early diagnosis of acute and gangrenous mastitis, leading to more effective therapies and more promising prognoses.

These results led to the following conclusions concerning the developmental

mechanisms of gangrenous mastitis caused by Ggam-negative bacilli.

In many cases, gangrenous mastitis occurs immediately after calving resulting in disuse or death due to its rapid course without allowing the sufficient treatment time. First, Gram-negative bacilli invade from the papilla into the cisternae of the mammary gland and the milk duct, where they proliferate and produce a large amount of endotoxin. The endotoxin flows out of the epithelia of the mammary gland into the circulating blood and induces intravascular coagulation syndrome by affecting the fibrinolysis system of blood coagulation and the complement system. Furthermore, it is considered that a direct action of the endotoxin causes tissue damage such as hemorrhage, necrosis and thrombus formation in the udder and the main organs, leading to systemic Shwartzman phenomena and, finally, death.

In addition, the following conditions for the easy invasion of bacilli into the mother's body may be properly cited as non-endotoxin developmental factors : stress caused by calving ; poor rearing environment ; fatty deposits in the liver associated with a high content of nutrients in the feed ; metabolic disturbances ; and latent mastitis.