Detection of Endotoxin in Affected Milk from Cows with Coliform Mastitis

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Peracute coliform mastitis is of great economic importance in the dairy industry [1], since the infection with coliform organisms and the following production of endotoxin leads to high mortality [2, 3]. When diagnosis of peracute coliform mastitis is given by the clinical signs and hematological and hemato-biochemical findings, misdiagnosis on the prognosis is common. The presence of endotoxin in blood plasma should be checked for the precise diagnosis of bovine peracute coliform mastitis, which is characterized as systemic endotoxemia and is accompanied by severe quarter inflammation [2, 3, 7]. However, the Limulus test is highly complicated and thus inadequate for field tests. The detection of the endotoxin in milk would be an alternative method effective for early prognostic diagnosis. In cows with peracute mastitis, there is a report suggesting the amount of the endotoxin in the milk is about five times larger as compared with that in the blood plasma [3]. In the present study, Limulus coagulation test (LCT) for qualitative endotoxin analysis was employed. This test requires no special instruments, and the time for the detection of the endotoxin is approximately 1 hour. Thus, it can be considered as an effective tool for the early prognostic diagnosis of peracute mastitis.

The purpose of the present report is to evaluate the utility of LCT for the detection of the endotoxin in the milk from cows with coliform mastitis.

Twenty-three Holstein-Friesian cows with coliform mastitis with various ages and in different stages of lactation were examined. The affected cows were further classified into 2 groups (peracute type: 14, acute type: 9) on the basis of clinical signs. The clinical signs in cows with peracute mastitis were serous secretion from the affected quarters, pyrexia (progressing to subnormal in some cases), depression, anorexia, dehydration and diarrhoea. In the cows with

acute type mastitis examined in the present study, dehydration and diarrhoea which are commonly observed in peracute cases were absent.

Foremilk samples from affected quarters were aseptically collected before treatment using sterilized pyrogen-free tubes. They were inoculated (0.05 ml) on 5% sheep blood agar plates and were incubated aerobically at 37°C. Recovered microorganisms were identified using procedures previously described [6].

The remainders of the samples were examined for endotoxin using a kit of LCT (Pre-gel: Seikagaku Kogyo Co.), which employs the gelation method [4, 5]. Limulus lysate was dissolved in sterilized 0.1 ml pyrogen-free distilled water and 0.1 ml of milk sample was added by 1.0 ml disposable pyrogen-free tuberculin syringe (Terumo Co.). The procedures for the endotoxin assay are shown in Fig. 1.

On the bacteriological examination of the milk, 23 strains of coliform organisms were isolated. Of all the strains, 11 were identified as *Escherichia coli*, 8 *Klebsiella pneumoniae*, 1 *K. oxytoca*, 1 *Proteus vulgaris*, 1 *Serratia marcescens* and 1 *Citrobacter diversus*.

Seven of the 14 cows with peracute mastitis were positive for the endotoxin and died within 9 days. Of the remaining 7 cows, 4 showed a trace of endotoxin. Of these 4 cows, one died, 2 were slaughtered and another one recovered. The remaining 3 endotoxin-negative cows all recovered (Table 1).

In the 9 cows with acute coliform mastitis, only 1 cow showed trace reaction in LCT, and the remaining 8 cows were all negative. All these endotoxin-negative cows had a benign prognosis except one which were slaughtered (Table 2).

For cows with peracute coliform mastitis, prognostic diagnoses have commonly been done based on clinical symptoms and the hematological and hemato-biochemical examinations. However, the present study has shown that the prognosis of this disease made based on those methods is unreliable, since some cases with

Fig. 1. Procedures for Limulus coagulation test.

severe leukopenia and a marked decrease of peripheral blood platelets and serum fibrinogen concentration recovered afterwards.

The results the present study suggest that LCT might be helpful in making the early prognostic diagnosis for cows with peracute coliform mastitis. Since the detection of the endotoxin in the milk of affected cows correlates well with the cosequent death, it is recommended that they are kept from any medication.

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要 約

牛大腸菌性乳房炎乳汁中からのエンドトキシンの検出 (短報): 安里 章 (北海道農業共済組合連合会家畜臨床講習所)——大腸菌性乳房炎罹患牛23頭 (甚急性14: 急性9) の発症時の感染乳汁中のエンドトキシンの検出を Limulus coagulation test (LCT) により実施した。その結果, 甚急性乳房炎では7頭が陽性でこれらの症例はすべて死亡したが, 痕跡と陰性を示した症例に死亡例はなかった。急性乳房炎では陽性牛はなく, 死亡例もなかった。以上のことから, LCT は牛甚急性大腸菌性乳房炎の早期予後診断に応用可能であると考えられた。

Table 1. Relationship between the results of Limulus coagulation test (LCT) and prognosis of cow with peracute coliform mastitis

LCT	Death	Slaughter	Cured	Total
2+	1	0	0	1
1+	6	0	0	6
Trace	1	2	1	4
Negative	0	0	3	3
Total	8	2	4	14

Table 2. Relationship between the results of Limulus coagulation test (LCT) and prognosis of cow with acute coliform mastitis

LCT	Death	Slaughter	Cured	Total
2+	0	0	0	0
1+	0	0	0	0
Trace	0	0	1	1
Negative	0	1	7	8
Total	0	1	8	9

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