Characteristics of Bovine Lymphoma Caused by Bovine Leukemia Virus Infection in Holstein-Friesian Dairy Cattle in Korea

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ABSTRACT : The frequency and distribution of lymphoma caused by bovine leukemia virus (BLV) infection in various organs were investigated. Lymphoma samples were obtained from slaughtered cattle or from cattle submitted to the National Veterinary Research and Quarantine Service, Korea. Thirty female Holstein-Friesian dairy cattle aged over three years with the BLV-associated lymphoma were studied. None of the Korean native cattle (Hanwoo) had lymphoma in this study however. Lymphoma tissues were gray to pink in color, soft in consistency, and bulged from the cut surface. In advanced lymphoma tissues, there was great variety in the appearance of involved structures due to hemorrhage, necrosis, and/or calcification. Neoplastic tissues were observed in lymph nodes in all lymphoma cases. Intestine (96.4%), heart (88.9%), stomach (73.1%), and diaphragm (62.5%) were frequently involved with lymphoma. However, there was no lymphoma detected in liver. Large neoplastic masses, sometimes reaching the size of over 20 cm, were found in the abdominal cavities. It is suggested that metastasis of lymphomas occurs mainly via lymph based on gross observations; neoplasia may have been initiated in the serosal surface of the lung, heart, peritoneum, and numerous hollow organs in the abdominal cavity. Also many organs in the abdominal and thoracic cavity were affected by neoplastic tissues simultaneously. Characteristics observed in this study could be used as criteria to differentiate BLV-associated lymphoma from other nodular lesions in the slaughterhouse and as fundamental data to make clear the mechanism of metastasis or pathogenesis of EBL. ***(Asian-Aust. J. Anim. Sci. 2005. Vol 18, No. 5 : 728-733)***

Key Words : BLV, EBL, Bovine, Enzootic, Gross Finding, Leukemia, Lymphoma

INTRODUCTION

Enzootic bovine leukosis (EBL) caused by the bovine leukemia virus (BLV) infection is characterized by the proliferations of neoplastic lymphocytes in peripheral bloods and/or various organs. There are three different disease forms on BLV infection: 1) BLV infection alone without any clinical expression; 2) increase of the absolute number of peripheral blood lymphocyte; 3) lymphoma; the common form in adults (Burney et al., 1978; Radostitis et al., 1994). Thirty percent of naturally infected cattle present polyclonal B-cell expansion in the peripheral blood (persistent lymphocytosis [PL]), and one to five percent of cows develop generalized B-cell lymphoma in five to ten years following BLV infection, with a corresponding variety of clinical signs according to the organ in which lymphoma developed (Chevallier et al., 1998; Schwartz and Levy, 1994).

Numerous studies have been made on the BLV and EBL in Korea (Son et al., 1968; Jun et al., 1982; Choi et al., 1992). Frthermore 54.2% in Holstein-Friesian dairy cattle was BLV-seropositive in recent report (Suh, 2004). However, few studies have been performed on lymphoma, the clinical form of EBL, until now (Lim, 1969; Lee et al., 1982), and the reports on characteristics of lymphoma in the naturally BLV-infected cattle are limited in Korea. It is the purpose of this study to assess the frequency and distribution of lymphoma in the BLV- infected cattle.

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MATERIALS AND METHODS

Animals

Samples were obtained from slaughtered cattle, and from cattle submitted for disease diagnosis to the Pathology division, National Veterinary Research Quarantine Service (NVRQS), Korea. Four lymphoma-suspected samples were collected at NVRQS diagnostic laboratory from January 1993 to June 2004 and 43 samples at abattoir from January 2002 to June 2004.

Gross observation of lymphoma

All part of carcass and the organs in the abdominal and thoracic cavities were carefully observed for the presence of lymphoma-like nodular lesion. However, superficial lymph node, spinal cord, and head were not examined in many cases, because they were routinely removed and discarded after post-mortem inspection in abattoir. Ante-mortem inspection was also not done in all cases.

Polymerase chain reaction (PCR) test

Nested PCR test was performed on all cattle with lymphoma-suspected gross lesion, as described earlier to detect *env* gene, coding for GP51 of BLV provirus (Ballagi-Pordany and Belak, 1996; Kobayashi et al., 2004; Renstrom et al., 2000). Table 1 shows the sequences of the primers for

Table 1. Oligonucleotide primers for the PCR amplification of BLV provirus

Oligo	Sequence	Position	Usage
OBLV1A	5'-CTTTGTGTGCCAAGTCTCCCAGAGTACA-3'	5,029[1]	1st PCR[2]
OBLV6A	5'-CCAACATATAGCACAGTCTGGGAAGGC-3'	5,442	1st PCR
OBLV3	5'-CTGTAAATGGCTATCCTAAGATCTACTGGC-3'	5,065	2nd PCR[3]
OBLV5	5'-GACAGAGGGAACCCAGTCACTGTTAACTG-3'	5,376	2nd PCR

[1] Gene bank accession number: K02120. [2] Product size: 440 bp. [3] Product size: 341 bp.

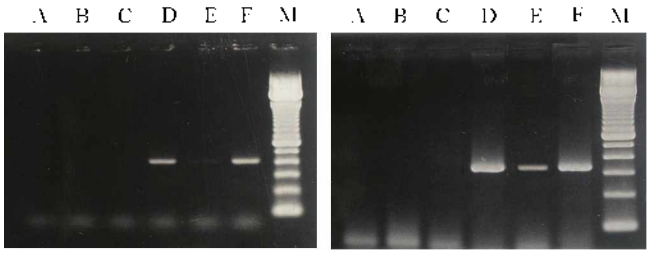

Figure 1 (1st PCR, left) and 2 (2nd PCR, right). Representative patterns of agarose gel electrophoresis of PCR amplified products to BLV provirus (A, B and C: BLV negative, D: BLV strong positive, E: BLV weak positive, F: BLV-infected FLK cell, M: DNA ladder).

BLV detection. Only the cattle which are positive both in PCR test and in gross finding are regarded as BLV-associated lymphoma in this study.

RESULTS

Detection of lymphoma

Among 47 heads of lymphoma-suspected, thirty cases (61.9%) were proved as BLV-associated lymphoma based on the PCR (Figures 1 and 2). All cattle which had BLV-associated lymphoma were over three year old and female Holstein-Friesian dairy cattle. Lymphoma in the Korean native beef cattle (Hanwoo) was not detected in this study. One cow which was negative in PCR test but had pathologically lymphoma lesion, was considered as sporadic bovine leukosis (SBL). Among the 16 cases which were BLV-negative in PCR test, fourteen cases were proved to be fat necrosis and two cases were granuloma caused by parasitic and bacterial infection, respectively.

Gross findings and organ involvement

Neoplastic tissues were white to pink in color. These masses were soft and mushy in consistency, and bulged from the cut surfaces. In severely progressed cases, there were great varieties in the appearance of involved structure and showed black or yellow pus-like foci on the cut surfaces by hemorrhages and/or necrosis. There were also multifocal white foci caused by calcification in necrotic mass especially in the central part of large neoplastic masses. The lymphomas were observed most frequently in the lymph node (100%) followed by the intestine (96.4%), heart (88.9%), and stomach (73.1%). Table 2 shows the results on the frequency of lymphoma within the various organs of cattle.

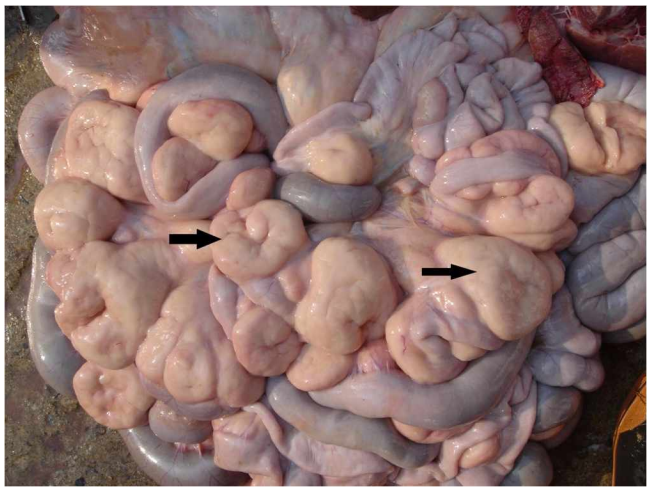

Figure 3. Small intestine. Note the enlarged white neoplastic mesenteric lymph nodes with central depression and coalescing pattern (arrows).

The affected lymph nodes were commonly found as enlarged masses. On the cut surfaces, normal corticomedullary architecture was not found. Big neoplastic tissues reaching the size of larger than 20 cm in diameter had developed by a conglomeration of several neighboring lymph nodes or by enlargement of one lymph node, especially in the abdominal cavity.

The abomasal wall, when involved, showed an uneven thickening of submucosa by neoplastic cells, particularly in the pyloric region. In few cases, multifocal mucosal ulceration was seen. The neoplastic tissues were also proliferated mainly in the submucosa of the nonglandular stomachs. There were two types of neoplastic changes in the intestine. One type was the neoplasia formed on the serosal surface of intestine. In the majority of infected animals, there were numerous masses on the serosal surfaces sized up to two to three cm in diameter. The other type was the lymphoma which was initiated in the mesenteric lymph nodes (Figure 3). In the former cases, it appeared that lymphoid cells initially proliferated in the serosa without any association with the intestinal wall but later infiltrated into the intestinal wall. The latter is firmer and whiter than normal lymphoid tissue. However, neoplastic tissues were developed in both of serosal surface and the mesenteric lymph node in many cases.

Table 2. Distribution of lymphoma followed by bovine leukemia virus infection in various organs

Case No.	Lymph node	Intestine	Heart	Stomach	Diaphragm	Kidney	Uterus	Lung	Others
99Q099	+	+	-	+	NE	-	NE	NE	
99D394	+++	+	+	+	NE	+	+	-	
00Q011	+	+	++	++	-	+	-	++	
02R010	+++	++	+	+	NE	++	-	-	
03R015	+++	+++	-	+++	+	-	-	-	
03R016	++	+	++	+++	-	-	-	-	
03R017	++	+	NE	NE	++	NE	NE	-	
03R018	++	+	+	+	+	+	+++	-	
03R019	+	+	+	-	-	-	-	-	
03R020	++	NE	+++	NE	NE	NE	NE	NE	
03Q129	+	-	+	-	-	++	+	-	
04R001	+++	++	NE	NE	NE	NE	NE	NE	
04R004	+++	NE	NE	NE	+++	+	-	-	
04R005	+++	+++	+	+	++	-	-	-	
04R006	+++	+++	+	++	NE	-	NE	NE	
04R007	+++	+++	++	+	+	++	NE	-	
04R008	+++	+++	+	++	+	+	NE	-	
04R010	+++	+	+	-	+	-	+++	-	
04R011	+	+	-	+++	-	NE	-	-	
04R012	+	+	-	-	-	-	-	-	
04R015	+++	+++	++	+	++	-	-	-	
04R019	+++	++	+++	++	+	-	++	-	
04R020	+++	++	+	+	++	+	-	++	Vagina
04R021	+++	+++	+	++	+	-	-	-	Spinal cord
04R023	++	+	+	-	++	NE	-	-	
04R028	++	+	+	+	+	-	-	-	Aorta
04R031	+	+	+++	-	-	-	-	-	Muscle
04R032	+++	+	+++	-	-	++	+	-	Muscle
04R035	++	+	-	+	-	-	-	-	
04R036	++	++	+	+++	++	+++	+	NT	Urinary and gall bladder
No. of examined cattle	30	28	27	26	24	25	23	23	
No. of cattle with lymphoma (percent)	30 (100.0)	27 (96.4)	24 (88.9)	19 (73.1)	15 (62.5)	11 (44.0)	7 (30.4)	2 (8.0)	

−, −−, −+−: Presence of lymphoma and severity of neoplastic lesion.
-: Absence of lymphoma. [1] Not examined.

Although firm white neoplastic masses were found in many organs, the most striking features were shown in the heart. The neoplastic masses invaded particularly right atrium. Normal cardiac muscle was replaced by neoplastic tissues and thickened to a varying extent. The involved endocardial surfaces and cut surfaces of muscle were white in color and had numerous bulging or polypoid masses on the endocardial surface of atrium and ventricle, which were sometimes easily detached from the endothelium (Figure 4). In one case, the neoplastic mass was found only in the epicardial fat without any neoplastic tissues in other parts of the heart. In some cases, there was fibrinous to fibrous epicardial inflammation.

Neoplastic masses were easily seen in the surface of the kidney as white nodules protruding out of the surface. Multiple white mottling or nodules of leukotic tissue were observed both in the cortex and in the medulla when sectioned sagitally (Figure 5). In many cases (54.5%), neoplastic tissues were limited at the renal hilus or serosal surface of the kidney, but not developed within the renal parenchyma. The wall of the involved uterus was thickened and sometimes showed nodular protrusions out of the mucosal surface. In one case, the two cm masses in diameter were detected on the mucosa of the vagina and urinary bladder (Figure 6) which protruded into the lumen.

Involvement of lymphoma in the lung was relatively rare. Neoplastic masses were observed in the connection with the surface of the lung. These masses were infiltrated into the adjacent tissues, especially into interlobular connective tissue. In all EBL cases, the hepatic tissue was free of lymphoma in this study. There was only one case in which neoplastic masses were found in the hepatic hilus.

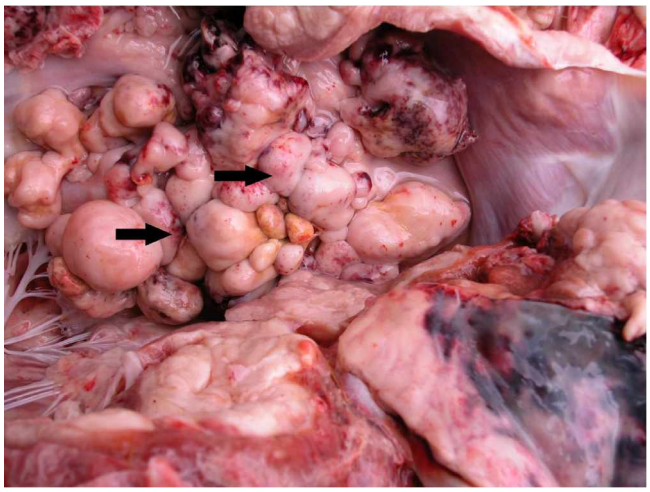

Figure 4. Heart. Note the nodular leukotic mass (arrows) bulging from endocardial surface of right atrium and ventricle.

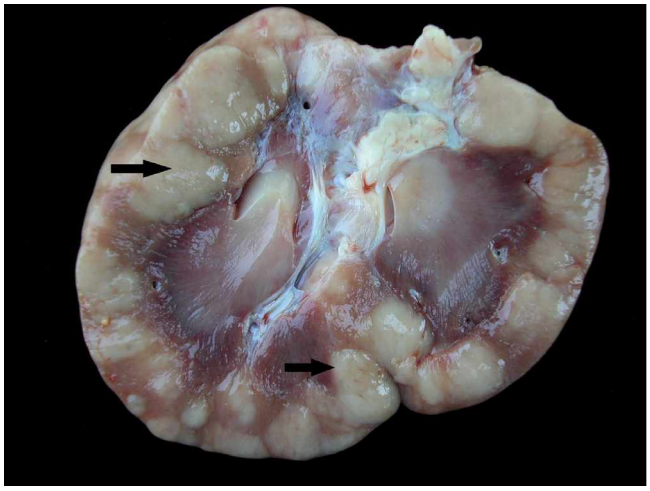

Figure 5. Kidney. Note the cut surface of sagittal section with white mottle (arrows) in the cortex and medulla.

but infiltration into the parenchymal tissues was not detected. The diaphragm was frequently involved with lymphoma. Both the abdominal and thoracic surfaces of the diaphragm were equally involved with variable sized mass (one to 15 cm in diameter). Pleural and peritoneal surfaces of the carcass were sometimes involved with lymphoma corresponding with diaphragmatic lesion. In one case, firm and white neoplastic mass was observed on the subdural space of the cervical vertebrae, attached to the spinal cord by fine fibrous connective tissue. There were neoplastic masses in the neck muscle in two cases.

DISCUSSION

Although the proportion of BLV-seropositive cattle have been increased in Korea (Kim et al., 1990; Shim et al., 1998; Suh, 2004), there are few reports on the bovine lymphoma; clinical form of BLV infection. Because

Figure 6. Urinary bladder. Note the numerous round neoplastic mass formed in the mucosal surface.

lymphoma is the most commonly detected disease from slaughter house (Bradley and Migaki, 1963), we were tried to detect the lymphoma mainly at slaughter house.

All the cattle which had lymphoma were over three years old Holstein-Friesian cattle. None of the Korean native cattle had lymphoma. One possible explanation is that most of the Korean native cattle reared for meat production are slaughtered at younger age than dairy cattle (Burney et al., 1987; Schwartz et al., 1994), which does allow for cattle to be infected with BLV and to develop lymphoma. Dairy cattle have many chances to contact with the BLV-infected blood by way of milking and iatrogenic procedures including vaccination and many kinds of treatment procedures (Johnson and Kaneene, 1992; Song et al., 2002). Also Korean native cattle may be genetically resistant to development of lymphoma (Olson et al., 1970; Lewin and Bernoco, 1986; Ramesha et al., 2002; Yeo et al., 2002). All lymphoma cases were females in this study. This may be due to the fact that males go to market at an early age except for a few retained cattle for breeding (Smith, 1965; Burny et al., 1988; Choy et al., 2001).

In general, neoplastic lesions in heart and hollow organs such as the abomasum and intestine were conspicuous with those in the lymph nodes accordance with previous reports (Smith, 1965; Valli et al., 1981). Invasion of neoplastic cells into the hollow organs was repeatedly observed to be merely anatomical progression from the serosa, and the same was true of the diaphragm and the abdominal and thoracic surface of carcass. In previous reports, neoplastic cells appeared to be interdependence between involvement of lymph nodes and serosa surfaces in the peritoneum and pleura (Bendixen, 1965; Smith, 1965). However, it looks that these were no interdependence between involvement of lymph nodes and serosal surfaces.

The most pathognomonic lesion was found in the heart, which was invaded in 88.9% of the individuals. This

finding was higher than that (54.6%) of Smith's report (1965), but lower than that (90.9%) of Ohshima et al. (1980). In the early stage forms, small neoplastic masses were limited and identified only in the epicardial fat of the atrioventricular junction (Marshak et al., 1962; Jarplid, 1964). The mass might be implanted from the serosal surfaces of thoracic cavity. The neoplastic cells within the initial mass may invade right atrium, and then migrate to the right ventricle and left side, as previously described (Ohshima et al., 1982). Also Smith (1965) considered that the neoplastic tissue was regularly at the base of the heart into the wall of the atria.

The kidney was moderately affected in bovine lymphoma by 44.0%. This is relatively similar to the previous reports described 15.7% to 45.5% by Smith (1965) and Ohshima et al. (1980). In many cases, only renal hilus was involved with lymphoma and parenchymal structure was normal. These neoplastic cells may be originated from its regional lymph node (Marshak, 1962). The lung involvement was not frequent and detected only two cases among 25 cases. Smith (1965) reported that lymphoma started at the peribronchiolar lymph nodes in the lung, spreading from these small aggregate of lymphoid cells which were presumably susceptible to the neoplastic stimulus. However, we postulate that neoplastic cells are launched to serosal surface of the lung via lymph and not via blood. It is also possible that neoplastic cells might be originated from implanted cells floating in the thoracic cavity.

The liver involvement of lymphoma varied between 0.9% and 54.5% (Smith, 1965; Ohshima et al., 1980; Valli et al., 1981). From the data presented here, there was no hepatic involvement in BLV-associated lymphoma. However, severe hepatic lymphoma lesion was also observed in SBL case detected in this study in accordance with previous report (Radostitis et al., 1994). Wu et al. (1999) reported that the spleen was not the organ initially responsible for the transformation of EBL lymphoma and was vulnerable to metastasis from peripheral blood. One case of the spinal cord involvement was due to being removed at the time of carcass processing. Then, only a few spinal cords were examined. It is argued that more spinal lesions might have been detected if more samples had been examined. In previous reports, relatively diffuse infiltration into perineural fat and heavy involvement of the sheath of the spinal nerves was described alike to our finding (Jubb et al., 1993).

It is suggested from this study that lymph might be the main route of metastasis of bovine lymphoma; neoplastic change was initiated in the serosal surface of the lung and heart, and many hollow organs in the abdominal cavity. Gross appearances and frequency of organ involvement observed in various organs could be used as criteria to detect BLV-associated lymphoma in the slaughter house and to differentiate grossly EBL from SBL. It is likely for future research to clarify the properties of genetic resistance of the Korean native cattle (HanWoo) to BLV infection.

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