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Distribution of the serum Ig G titers to whole cell and leukotoxin of *Mannheimia haemolytica* A1 in Holstein and Korean indigenous cattle slaughtered in abattoir

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Abstract

A total of 419 slaughtered cattle were used to investigate the serum Ig G titers to the *Mannheimia haemolytica* A1 whole cell and leukotoxin recognized with important virulence factor in bacterial pathogenesis. Data obtained in this study were represented with average absorbance ± standard deviation. Serum Ig G titers were detected with the ranges from 0.1 to 0.5 at 490nm. Whole cell titers were higher than leukotoxin antibody on the whole. Antibody titers of slaughtered cattle between races, ages have no significant difference but gradual decrease under aging in dairy cow for whole cell (decline mean titer from 0.29 to 0.27 according to age) was undertaken. Holstein bulls shipped from Seoul province had a significantly lower Ig G titers than those from another ones ($p<0.05$).

Key words : *Mannheimia haemolytica*, Antibody titers, Leukotoxin

Introduction

Mannheimia haemolytica A1 is an opportunistic bacterial pathogen associated with shipping fever and enzootic pneumonia[1,2]. In association with stress conditions such as virus infection, change of temperature, shipping or change of feed, *M haemolytica* can cause an acute fibronecrotizing pleuropneumonia and subsequent death[3-6]. Therefore, economical loss is very high. This bacteria produce several virulence factors; leukotoxin(LKT), capsular polysacchride(CP), and lipopolysacchride(LPS). These antigens play major roles of bacterial pathogenesis and are used as vaccine candidates to prevent the disease[7-11]. Of virulence factors, LKT, pore forming cytolysin, has a biological effect only ruminant leukocytes, for example, alveolar macrophage and induce influx burst of neutrophil and impair pulmonary defence mechanism[5,7,11-13]. It has been recognized that

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CP is resistant to phagocytes and complement lysis[7-9]. LPS enhance cytolysis and inflammatory cytokine induction and show endotoxic activity in a similar manner of other gram negative bacteria[10,14]. With infectious conditions, optimal level of Ig G to important virulence factors is more essential to protect the disease and also maintained for a given period of time in serum. Accordingly, Intensity of serum antibody for these antigens could be indicate the infection status and infer the protective potential for individuals. In order to examination of the serum Ig G titers, indirect enzyme linked immunosorbent assay(ELISA) was used in this study. ELISA method is widely used to detect the specific antigen and antibody in unknown samples[9,15-17]. The objectives in this study were to detect the serum Ig G level to whole cell and leukotoxin of *M haemolytica* A1 and compare the serum Ig G titers which were distributed by slaughter ages, sex and shipping area in holstein and korean indigenous cattle.

Materials and Methods

Animals

Three hundred and sixty five Holstein and Korean indigenous cattle of fifty four shipped from Seoul, Incheon, Kyoungki, Chungnam, Chungbuk and Gyeongbuk province were slaughtered from July to August in abattoir. Blood was obtained from jugular vein after death immediately and incubated for 3hrs to separate serum. After centrifuge at 3,000 ×g for 10min, supernatant was stored at -60℃ deep freezer until assay. Cattle was assorted by the ages, sex and race according to the slaughter applications. The approximate age of dairy cow were estimated with the number of a milk (deciduous) teeth.

Antigens preparation

Mannheimia haemolytica A1 was grown in tryptic soy broth(Difco, USA) for overnight. Culture broth was innoculated into RPMI-1640 medium(Sigma chemicals, USA) and further incubated for 6hrs to logarithmic phase. Whole bacteria was counted with standard plate count technique and added formaldehyde to be 0.3% concentration and incubated overnight. Bacterial cell was collected by centrifugation at 6,000 × g for 30min and washed 3 times with phosphate buffered saline(pH 7.2). Whole cell antigen was stored at 4℃ until use. Culture supernatant was filtered by 0.2㎛ milipore (Milli-Q, USA) and precipitated with ammonium sulfate. Sediment was dialyzed with against pyrogen-free distilled water and stored at -20℃. Protein concentration was determined by a commercial protein assay kit (BCA kit, PIERCE, France).

Indirect ELISA

Antibodies of the Ig G binding the whole cell and leukotoxin were assayed using an indirect ELISA. All reagents were adjusted by separate checkerboard titrations. Leukotoxin and whole cell were coated to well of 96 well plates, 20㎍ and 4.5×10^8CFU, respectively per well in 100㎕ 0.06M carbonate buffer(pH 9.6) for 2 hrs at 37℃. Following a wash step, plates were blocked for 1hr with 2% gelatin solution in phosphate buffered saline containing 0.05% Tween 20(PBST) and used as a wash solution in later step. After washing, 100㎕ of bovine serum diluted with 1 : 2 was reacted with antigen for 1hr at 37℃. Following a wash step, bound antibodies were detected using mouse monoclonal antibody to bovine Ig G(Sigma,

USA) 1 : 1000, incubating 37℃ for 1hr. Following wash, the mouse monoclonal antibody was detected using an horseradish peroxidase conjugated goat-antimouse Ig G, 1 : 2500 for 1hr incubation at 37℃. After washing, enzyme activity was assayed using SigmaFAST® tablet(O-phenylenediamine, OPD). Negative control serum was kindly gifted from bacterial division of National Veterinary Quarantine Service(NVQS). Optical density was detected at 490 nm. Antibody level was expressed as optical density of test serum minus the optical density of negative control.

Statistics

Statistical analysis was used to analyze mean titer differences between ages, sex or race by F-test. With the ANOVA(Analysis of Variances) model, the differences were determined with level of antibody titers according to shipping area. Student t-test was used to determine the difference of means between provinces

Results

Antibody response to *M haemolytica* whole cell

In case of comparison of the antibody levels between sex and race, holstein cattle were detected with a mean OD_{490} value of 0.28 ± 0.07(female), 0.29 ± 0.06(male) and 0.3 ± 0.04(male), 0.3 ± 0.05(female) in korean indigenous cattle (Table 1). In holstein cow, Ig G titers were gradually declined with the range from 0.29 ± 0.07 to 0.27 ± 0.07 follow increasing age(Table 2). In regional Ig G distribution of holstein bulls, cattle shipped from Seoul area had significantly lower Ig G titer than those from other provinces(Table 3). For regional differences of Korean indigenous cattle, the cattle from Incheon were 0.31 ± 0.04, represented with the most high titer than 0.30 ± 0.05 of Kyoungbuk and 0.28 ± 0.04 of Kyoung-ki province(Table 4).

Antibody response to *M haemolytica* leukotoxin

Holstein cow and bull have shown a same titer a mean OD_{490} value of 0.26 ± 0.06. Korean indigenous female and male cattle were detected with 0.26±0.04, 0.30 ± 0.05 respectively(Table 1). In accordance with the ages, antileukotoxin titers were reduced from 2 to 4 years old but slightly increased in 5 years cow. In survey of holstein bulls, the cattle shipped from Seoul area had a significantly lower Ig G titer than those from other provinces(Table 3). For regional differences of Korean indigenous cattle, Kyoungki was 0.25 ± 0.02, showing lower than 0.28 ± 0.05 of Incheon and 0.28 ± 0.01 of Gyeongbuk provinces(Table 4).

Table 1. Comparison of the serum antibody titers between Holstein and Korean indigenous cattle

Race	Sex	No of cattle	Absorbance(Mean±SD)	
			Whole cell	Leukotoxin
Holstein	F	192	0.28 ± 0.07	0.26 ± 0.06
Holstein	M	173	0.29 ± 0.06	0.26 ± 0.06
Korean indigenous cattle	F	31	0.30 ± 0.04	0.26 ± 0.04
Korean indigenous cattle	M	23	0.30 ± 0.05	0.30 ± 0.05

Table 2. Serum antibody distribution of dairy cow according to ages

Ages (year)	No of cattle	Absorbance(Mean ± SD)	
		Whole cell	Leukotoxin
2 ~ 3	36	0.29 ± 0.07	0.26 ± 0.05
3 ~ 4	46	0.28 ± 0.06	0.25 ± 0.06
> 5	110	0.27 ± 0.07	0.26 ± 0.05

Table 3. Regional antibody titers of Holstein bulls to whole cell and leukotoxin

Province	No of cattle	Absorbance(Mean ± SD)	
		Whole cell	Leukotoxin
Kyoungki	59	0.31 ± 0.06	0.28 ± 0.05
Chungnam	14	0.29 ± 0.05	0.24 ± 0.05
Chungbuk	20	0.29 ± 0.08	0.25 ± 0.07
Incheon	40	0.29 ± 0.06	0.26 ± 0.05
Seoul	20	0.25 ± 0.07*	0.22 ± 0.07*
Gyeongbuk	20	0.27 ± 0.06	0.24 ± 0.05

* : Significantly lower ($p<0.05$) than mean titers of other provinces

Table 4. Distribution of regional antibody levels for Korean indigenous cattle

Provinces	No of cattle	Absorbance(Mean ± SD)	
		Whole cell	Leukotoxin
Gyeongbuk	24	0.30 ± 0.05	0.28 ± 0.05
Incheon (Kangwha-gun)	15	0.31 ± 0.04	0.28 ± 0.01
Kyoungki	15	0.28 ± 0.04	0.25 ± 0.02

Discussion

Pneumonic pasteurellosis is a well known disease of ruminants worldwide[5,6]. It has been implicated that *Mannheimia haemolytica* cause of fibrinous pneumonia, called as enzootic pneumonia in young calves and shipping fever in feedlot cattle[1,2,18]. A lot of efforts to prevent and reduce the outbreak of disease have been made a trial by many researchers[8,16-19]. Especially, induction of the serum antibody to leukotoxin(LKT), lipopolysaccharide(LPS) and capsular polysacchride(CP) would be contribute to reduce the symptoms and prolong the time of outbreak[9,15-17,19,20]. Therefore, the detection of serum Ig G of farm cattle could predict the preventive capability against the infection. In our experiment, serum Ig G titers to whole cell(WC) and leukotoxin(LKT) were detected in Holstein and Korean indigenous cattle shipped in abattoirs to find out whether the differences were between ages, sex or area. In comparison of Holstein and Korean indigenous cattle, there was not significant differences but antibody titers of Korean indigenous cattle were generally higher than those of Holstein. Confer et al[15] hypothesis that the variation of antibody degree could be varied by the outbred and ages of cattle. For the detection of antibody to dairy cow in accordance with aging, antibody titers to WC were reduced following ages but, in case of LK, the cow of above five year were slightly increased rather than those of 2 - 3 years cattle. Although simple comparison without consideration of different area or rearing circumstance and so on was carried out, Ig G titers to both WC and LKT in dairy cow shipped to abattoirs generally were shown to incline antibody reduction follow as aging. In preliminary study, we examined about the lung consolidation used by method of Brogden et al[8] to know wether there was relation between lung lesion and antibody titer but no relation to slaughtered cattle (data not shown). Reeve-Johnson[21] showed that the clinical scores of the calves were correlated with the extent with of lung

consolidation. Frank[1] and Frank et al[3] indicated that healthy cattle can carry *P haemolytica* at undetectable levels for long periods of time and isolation rates of *P haemolytica* were few at the farm but markedly increased at feed yard. Also, Hodgin and Shewen[9,17] indicated that antibody titer was naturally increased in dairy cattle on the fall season which is changing the climate. In Korea, Holstein bull was generally slaughtered within two years old for the purpose of meat production. With the infection of pneumonia, the reduction of growth rate and feed coefficient cause a economical damage at the farm. Our results showed that the Holstein bulls from Seoul area had a significantly lower antibody titers than those shipped from the other provinces to both antigens. In a similar to Holstein bull, Korean indigenous cattle showed a regional difference for antibody titer but not significant. This results could probably be attributed by the geographical characteristics of which the farm located in Seoul were generally small and difficult to contact with another ones. If these cattle were infected with *M haemolytica*, respiratory tract disease could have developed with serious symptoms in animals. Frank et al[16] demonstrated that respiratory tract disease was significantly related to the farm of origin and was inversely related to the *P haemolytica* serum titers at the farm. Vaccination could also reduce the frequency of colonization of upper respiratory tract by *P haemolytica*. In our experiment, we detected the Ig G titer to WC and LKT from the Holstein and Korean indigenous cattle slaughtered in abattoirs and realized that differences of antibody levels between the regions were significant but not in the sex and race.

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