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A Study on Leptospiral infection of Cows by Blood Culture and Microagglutinin Test of Serum in A Korean Rural Area

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Abstract

The study on leptospiral infection among cows was carried out to find out possible role of this domestic animal as an infection source for humans, and the magnitude of economic loss due to abortions caused by leptospiral infections among the domestic animal.

Bood samples of 459 cows in May and 446 heads in November 1989 were collected from 19 villages of Kyunggi Province. These samples were cultured and serologically tested.

Only one strain of leptospires, most strongly reactive to Pomona antibody, was isolated from duplicated cultures of 985 cow blood samples in EMJH medium. Serological test by MAT with seven reference strains and three locally isolated strains was positive in 12% of the sample in May and 26% in November; positive rate increased slightly as the age of cows increased and male cows had higher positive rate, however, there was on difference in positive rate by type of cow and area. There was no statistically significant association between abortion history among cows(one yearperiod) and positive MAT.

Thus it was concluded that the leptospiral infection of cows may play a role for human infection, and the leptospiral infection of cow may not be the major cause of abortion although further study is necessary for definite conclusion.

Introduction

In Korea epidemic pulmonary hemorrhagic fever, as a new clinical syndrome by experienced clinicians since 1975, has been occurring sporadically from year to year yet without identification of the cause despite the continuous efforts.[8]

In the middle of September 1984 following flood there was another epidemic of the disease, still sporadic in nature but resulting in several deaths from the disease.

One of the authors above(Kim) was commissioned at that time to investigate the cause of the epidemic and it was proved to be leptospiral infection through a series of field and laboratory studies.[7]

Infection of domestic animals by leptospires is an important problem in two aspects; one is as a possible source of human infection and the other as an economic loss due to frequent abortion of infected cow.[3,5]

This study of leptospiral infection of cows was carried out preliminarily to determine a possible role of the cow as a source of sporadic human infection, and

Table 1. Strains Used for MAT

Serogroup	Serovar	Strain	Source
Autumnalis	autumnalis	Akiyama A	CDC
Bataviae	bataviae	V Tienea	CDC
Icterohaemorrhagiae	ndahambukuje	Ndahambukuje	pasteur
Louisiana	louisiana	LSU 1945	pasteur
Pomana	pomona	Pomona	CDC
Sarmin	sarmin	Sarmin	pasteur
Sejroe	hardjo	Hardjoprajitno	pasteur
Tarassovi	tarassovi	Mitis Johnson	pasteur
6p-058-2(c. canicola)*			
2-18-111-2(I. copenhageni)*			
87K-71-7(I. lai)*			

* Localy isolated starains in Korea.

the magnitude of economic loss owing to abortions caused by leptospiral infection among the pregnant cows.

Materials and Methods

Area Characteristics : The area studied is an ordinary and typical rural area covering 31.7㎢. There were 1,097 households with 4,315 population. At the time of the study the total number of cows being raised was 2,172 heads.

Sampling Procedure : Blood samples of 495 cows in May and 446 heads in November of 1989 were collected aseptically in vacutainer, labelled with code number of the cow, sex and age, packed in board box and transported to the laboratory on the day of collection.

Culture and Serological Test : Blood sample in vacutainer was centrifuged at 3500 rpm for 30 minutes and then serum was separated and kept in refrigerator for MAT(microagglutination test). The sediments of the blood were inoculated to EMJH media in two to three drops in duplicates and kept in 30℃ incubator. The culture tubes were examined for ring formation macroscopically, and the inuculum under dark field with × 400 microscope for leptospires in every week for two months.[4)]

Eight standard strains and three locally isolated strains of leptospires from human were used as antigen for the MAT. Separated sera were inactivated in 56℃ water bath for 30 minutes before the test. The criteria for positive test was 1 : 80.[4)]

Table 1 presents the strains of antigen used in this study. These locally isolated strains are identified as *C. canicola, I. hemorrhagiae* and *I. lai* by *cross absorption test*, The standard strains were obtained from either Pasteur Institute of France or CDC of U.S.A.

Results & Discussion

Isolation of Leptospires : Only one strain of leptospires was isolated out of 905 cow blood samples cultured. This one strain isolated from the May sample reacted most stronly with Pomona antiserum.

Positive Rate of MAT : The blood samples collected in November showed much higher positve rate than that collected in May, 26.2% versus 12.8%. This finding corresponds with the seasonal difference in positive rate among human population. The positive rate by serogroup of the antigen used revealed rather an evean distribution as shown in Table 2.

In Europian countries and American continents several serogroups of leptospires had been isolated such as *L. sejroe* and *L. pomona* most frequently.[4,5)] This was the first case of leptospiral isolation from a cow, however, in Korea. A few seroepidemiological

Table 2. Positive Rate of MAT on 905(1st 459, 2nd 446)Cattle Sera by Serogroup of Antigen

Serogroup	No. positive		Positive rate (%)	
	1st Survey (May) : 459	2nd Survey (Nov.) : 486	1st Survey (May)	2nd Survey (Nov.)
Autumnalis	3	4	0.7	0.9
Icterohaemorrhagiae	20	11	4.3	2.5
Pomona	ND	13	ND	2.9
Sarmin	–	9	–	2.0
Sejroe	–	14	–	3.1
Tarassovi	3	20	0.7	4.5
6p-058-2(C. canicola)	15	16	3.3	3.6
2-18-111-2(I. copenhageni)	5	8	1.1	1.8
87K-71-7(I. lai)	9	22	2.6	26.2
Total	55	117	12.0	26.2

Table 3. Seropositive Rate by Age

Age	No. tested	No. positive	Positive rate (%)
1mm~3mm	17	2	11.8
4mm~12mm	208	41	19.7
13mm~3y	290	49	16.9
4y~6y	267	39	14.6
7y~9y	64	18	28.1
10y	11	0	0.0
Unknown	48	11	22.9
Total	905	160	17.7

Table 4. Seropositive Rate by Sex

Sex	No. tested	No. positive	Positive rate (%)
Male	54	14	25.9
Female	787	138	17.5
Unknown	64	8	12.5
Total	905	160	17.7

studies on cows in Korea showed a wide range of positive rate; for example Chai[2] reported 3.8%, Suh *et al*,[3] 8.6% and Suk *et al*, 13.1%[11] On the other hand the seropositive rate of cows in tropical countries like Malaysia and Thailand was reported to be much higer than the positive rate we obtained. The positive rate among Malaysian cows was 27%[1] and that of Thailand was 40.5%.[6]

Table 3, 4 and 5 show the positive rates of cows tested by age, sex and a kind of cow. There was a tendency of increase in positive rate as cows grow older. Male cows had significantly higher positive rate, but there was on difference in positive rate between dairy cattle and Korean cattle.

Table 5. Result of Serological Test for Leptospirosis in Dairy and Korean cattle

Animals tested	No. tested	No. positive	Positive rate (%)
Deiry Cattle	628	115	18.3
Korean Cattle	213	37	17.4
Unknown	64	8	12.5
Total	905	160	17.7

Table 6. Result of MAT and History of Abortion

MAT	Abortion		Total
	Yes	No	
Positive	17	143	160
Negative	53	692	745
Total	70	835	905

Table 6 is two by two table to see association between abortion history of the past one year and the result of the MAT. in this study failed to demonstrate the correlation of leptospiral infection to the history of abortion.

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한국의 한 농촌지역에 있어서 혈액배양과 혈청검사에 의한 소의 렙토스피라 감염 조사 연구

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경기도의 19부락에서 1989년 5월에 459두와 그해 11월에 446두의 소를 대상으로 하여 렙토스피라 감염유무를 조사하였다.

혈액배양에서는 포모나 항체에 강하게 반응하는 한 균주만이 검출되었다. 몇개의 표준균주를 사용한 혈청검사에서는 5월에 12%, 11월에 26%의 양성율을 나타내었다. 년령이 증가함에 따라 양성율이 높아졌으며 숫소가 암소보다 높았다. 혈청검사 양성과 소의 유산력과는 유의적인 관계가 인정되지 않았다.