

# Serologic Studies of Japanese Encephalitis in Domestic Animals and Chickens

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## Introduction

Since the report in 1947 by Sabin et al<sup>1)</sup> on the first isolation of Japanese encephalitis (JE) virus in Korea, outbreaks of the disease have occurred every year, occasionally in epidemic proportions<sup>2)</sup>. In Japan the ecology of the disease has undergone intensive investigation<sup>3-11)</sup>, and considerable knowledge of vector relationships and natural range of hosts has been accumulated. Yet information as to specific natural history of JE in Korea is meager, although it has generally been assumed that there are basic similarities in the ecology of the disease in these two geographical areas. A phase of studies attempted in this report, therefore, is to find occurrence rates of hemagglutination-inhibition (HI) antibodies among domestic animals and chickens in or near Seoul area and to discuss the possible influence of immunologic factors upon the disease spread.

## Materials and Methods

**Serum samples.** Blood samples were collected in or near Seoul area during the period of 4 weeks

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from mid-October to early November, 1961. These included 54 horses, 92 Korean cattle, 37 hogs, 48 dogs and 136 chickens. Horses, consisting of racers and riding horses including one colt of approximately 6 months of age, were bled from jugular vein. Serum samples from cattle and hogs were obtained at the municipal abattoir, Seoul. Estimated age of cattle ranged from 3 to 5 years, and that of swine from 6 to 15 months. Dogs, mostly of police dogs, were less than one year of age with the exception of one dog over 2 years of age. They were bled by femoral or jugular venipuncture. Chickens, of 3 to 6 months of age, were bled by heart puncture prior to slaughter at Namdaemoon market in Seoul. After removal from the clot, serum sample was stored in a frozen state until use.

**Preliminary treatment of serum.** All serums were inactivated at 56°C for 30 minutes. It has been known that serums of dogs, swine and cattle often contain normal agglutinin against avian cells<sup>12)</sup>, and cattle serums also possess conglutinin<sup>13)</sup>. Consequently, the dog and swine serums that had been found to cause slippery pattern of hemagglutination and all of cattle serum samples were incubated at 37°C for 1 hour with 0.2 ml to 0.3 ml of packed adult goose cells per ml of serum and then centrifuged at 400G for 10 minutes. The supernates as well as unabsorbed serum samples were subject to acetone extraction twice.

**HI test.** The methods of HI test employed were essentially similar to those described by Buescher

et al<sup>14</sup>) and Scherer et al<sup>15</sup>). Antigen was prepared with strain coded as M5/596<sup>16</sup>) and used as dilutions of 8th passage suckling mouse brain in Hanks' balanced salt solution. In the earlier parts of the tests, the antigen diluent contained 1 per cent normal horse serum as stabilizing proteins. In the course of experiment, however, it was found that substitution of horse serum with 1 to 3 day old normal chick plasma exerted no significant influence upon the titers of antigen. Subsequently, the use of chick plasma as antigen stabilizer followed. For testing chicken and horse serums, 1-3 day old chick red cells were used, while adult goose cells were selected for use in testing cattle, dog, and pig serums, simply because large amounts of cells could be readily obtained for preliminary absorption. Highest serum dilutions tested [were 1:320. Inhibition of hemagglutination by a serum dilution of 1:10 or greater was considered as positive.

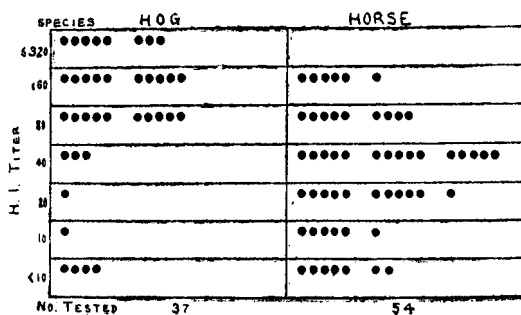
### Results

The results of HI tests are presented in the Table I, which shows that 47 of 54 horses (87%), 82 of 92 cattle (89%), 33 of 37 swine (90%), 8 of 48 dogs (17%) and one of 136 chickens were found to develop demonstrable HI antibodies in their serums. Figures 1, 2, and 3 illustrate the distributions of HI antibody levels of hog and horse, dog and cattle, and chicken respectively. The peak distribution of swine antibody titers occurs at the levels of 1:80 and 1:160, whereas that of horse titers is observed at the dilution of 1:40. In contrast, the concentration of cattle sample titers appears in lower dilutions, nearly half of them being at the dilution of 1:10, which simulates the pattern of human titers<sup>17</sup>). The geometric means of HI antibody titers were calculated for each group of pigs, horses, and cattle, and are illustrated on log<sub>2</sub> scale in Figure 4. On the assumption that a difference of 2 or more on this scale can be regarded as significant on an empirical basis, it appears that differences in pig and horse (1.3) and in horse and cattle (1.1) are inconclusive, whereas significant difference (2.4) exists between pig and cattle titers.

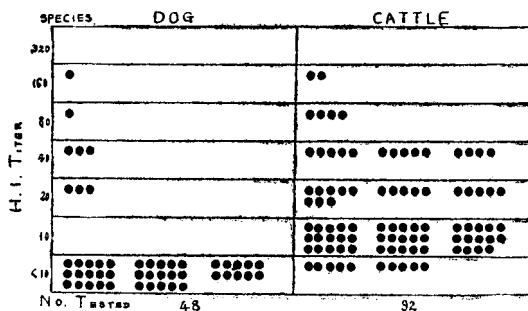
**Table I.** Occurrence of Hemagglutination-Inhibition Antibodies against Japanese Encephalitis Virus among Domestic Animals and Chickens.

	No. Tested	No. Positive ※ (% positive)
Horse	54	47 (87%)
Cattle	92	82 (89%)
Hog	37	33 (90%)
Dog	48	8 (17%)
Chicken	136	1

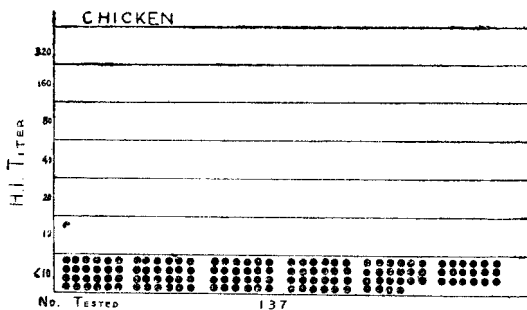
※ Positive=Inhibition of Hemagglutination by a Dilution of Serum Greater than or Equal to, 1:10.



**Fig. 1.** JE HI Antibody Levels of Hog & Horse Sera



**Fig. 2.** JE HI Antibody Levels of Dog & Cattle Sera



**Fig. 3.** JE HI Antibody Levels of Chicken Sera

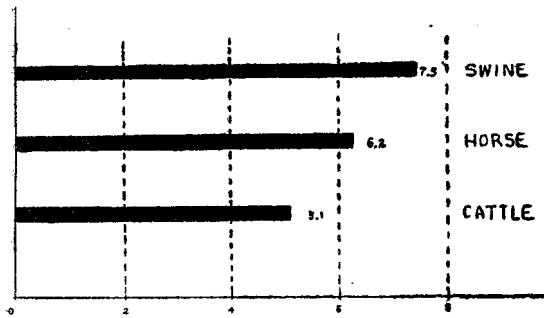


Fig. 4. Geometric Means of HI Titers to JE Virus of Swine & Cattle Serum Samples on a Log<sub>2</sub> Scale

### Discussion

Limited serologic studies by Deuel and Sabin<sup>18</sup>) revealed that almost 100 per cent of horses, swine, and cattle, but none of 12 chickens, in Kunsan and Seoul areas contained neutralizing (N) antibodies to JE virus. Subsequent investigations have generally confirmed those of Deuel et al and show high prevalence rates of N or HI antibodies among domestic animals in Korea<sup>19,20</sup>). In contrast to this finding is that of that of Lee et al<sup>19</sup>), who found that prevalence rates of N antibodies in chickens of southern provinces of Korea in 1956 were increased from as low as 4.4 per cent in July to over 40 per cent during the season. Perhaps these differences are due to localities, ages of chicken or other ecologic circumstances to which sampled chickens had been exposed. Unfortunately, however, neither the age of chickens nor detailed neutralization indices were indicated in their report. Observations made in Japan by the 406th Medical General Laboratory show that 10 to 20 per cent of the chicks had maternal antibodies to JE virus by HI test<sup>21</sup>) and that approximately 10 per cent of the chicks demonstrated neutralizing maternal antibodies<sup>22</sup>). In view of large yearly turnover of chicken populations in Korea, certainly more extensive and critical consideration should be given to the role of domestic birds as potentially important or unimportant natural hosts of JE virus in Korea.

Since all chickens and most of pigs and dogs tested were less than one year of age, it is obvious

that antibodies engendered in these hosts were seasonal antibodies resulting from exposures to a single epidemic season of that year. Thus, the findings that 90 per cent of hogs, 17 per cent of dogs, and less than 1 per cent of chickens had HI antibodies seem to suggest the high exposure frequencies of swine to transmitting mosquitoes per year, considerably lower infection rate in dogs and practically negligible degree of vector-domestic bird feeding association near Seoul. Accordingly these results strongly suggest that pigs, with their large yearly population turnover, play an important role in the ecology of JE near Seoul. It also seems that chicken's contribution to the infection of mosquitoes near Seoul is insignificant despite their large yearly population turnover. There is no published report available of attempts to demonstrate antibody levels of JE in dogs in Korea, although Hammon et al<sup>23</sup>) reported that 67 per cent of dogs tested in Guam harbored neutralizing antibodies.

Significance of cattle or horse as possible reservoir host is not so apparent as for swine. Since large proportions of adult cattle and horses with their smaller yearly population turnover are to carry demonstrable antibodies, the possibility of these larger vertebrates as primary reservoirs seems to be eliminated. Much tenable is the belief that these animals merely constitute secondary hosts and play insignificant role in rapid viral build-up during the pre-epidemic season. The possibility that antibody levels detected in this study had at least in part resulted from anamnestic antibody responses, like in monkeys<sup>16</sup>) and possibly in humans<sup>17</sup>), may deserve further attention.

It is of interest to note that the geometric means of swine HI titers are the highest, the difference between titers of swine and cattle being 2.7 on log<sub>2</sub> scale. It is not known, however, whether the greater magnitude of antibody responses in swine after one epidemic season need to imply innate susceptibility related to host species against JE virus.

### Summary

Occurrence of hemagglutination-inhibition (HI) antibodies to Japanese encephalitis (JE) virus was

studied among various domestic animals and chickens in or near Seoul area. The times of sample collection were mid-October to early November, 1961. Horses, consisting of 53 adults and one colt, were bled from jugular vein. Blood serum samples from cattle and hogs were collected at the abattoir, Seoul, their estimated ages being 3 to 5 years and 6 to 15 months respectively. Most dogs bled were less than one year of age. Blood samples of chickens, mostly of broiler age, were obtained by cardiac puncture prior to slaughter. The results obtained are summarized as follows:

1. Of 54 horses, 47(87%) were found to possess demonstrable HI antibodies.
2. Eighty-two of 92 cattle serum samples (89%) tested were positive.
3. HI antibodies were detected from 33 of 37 swine samples(90%), the levels of titer being the highest of all animal groups tested.
4. Tests of 48 dog serum samples revealed 8 positive samples(17%).
5. In regard to chicken samples, only one of 136 had a low (1:10) HI titer.
6. Implications of these findings in the ecology of JE near Seoul were discussed.

國文抄錄

家畜 및 家禽에 있어서의 日本腦炎에  
關한 血清學的 調査

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서울 및 그 近傍의 各種 家畜 및 家禽에 있어서의 日本腦炎 바이러스에 對한 血球凝集抑制抗體 (HI抗體)의 存在를 調査하기 위하여 1961年 10月 中旬부터 11月上旬 까지 期間에 54頭의 馬, 92頭의 韓牛, 37頭의 豚, 48頭의 犬 및 136首의 鷄에서 各己 血清을 採取하였다. 말은 53 頭의 成熟 乘馬 및 競馬와 1頭의 幼馬에서 頸靜脈穿刺로 採血하였다. 소와 돼지의 血清은 市內馬場洞屠場에서 採集하였으며 推定年齡은 소는 3 내지 5歲, 돼지는 6 내지 15개월 이었다. 개는 6개월 내지 2年の 訓練犬의 頸 또는 前膊頭靜脈血이었다. 닭은 南大門市場에 集結한 嬰鷄였으며 屠殺直前에 心穿刺한 것이다. 檢査에 있어서는 개와 돼지의 血清의 一部 牛血清全部를 壓沈成窩血球로

1回 吸收하였다. 全血清은 에세톤으로 2回 抽出하였다. 實驗結果를 要約하면 다음과 같다.

- 1) 54頭의 馬中 47頭(87%)의 血清에서 H.I.抗體를 檢出하였다.
- 2) 소 92頭中 82頭(89%)에서 血清 H.I. 抗體를 證示하였다.
- 3) 豚血清 37中 33標本(90%)에서 H. I. 抗體가 出現됨을 보았다.
- 4) 개에서는 48例中 8例 (17%)의 血清이 陽性으로 나타났다.
- 5) 닭에 있어서는 136首中 單1首만에서 低價의 血清H. I.抗體를 檢出할 수 있었다.
- 6) 上記 所見이 日本腦炎바이러스의 生態學에 있어서 暗示하는 바를 考察하였다.

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