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# Infection of herons and domestic fowls with Japanese encephalitis virus with specific reference to maternal antibody of hen (epidemiological study on Japanese encephalitis 26

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# Infection of herons and domestic fowls with Japanese encephalitis virus with specific reference to maternal antibody of hen (epidemiological study on Japanese encephalitis 26*

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**Abstract**

In order to ascertain whether black-crowned night herons (BCNH), white heron (Plumed Egrets (PE)) and domestic fowls are infected by JE virus and they serve as infection source ofJE, hemoagglutination inhibiting antibody and its 2·ME sensitive antibody in the sera of these birds were determined. Physico-chemical nature of fowl's antibody of JE produced by natural infection and their maternal antibody in the sera of chicks were examined. The results are briefly summarized as follows. 1) As to the herons captured in Tsudaka Town, two out of six adult night herons and three out of the four chicks showed positive HI reaction. On the other hand, HI reaction in the sera of two adult white herons and three chicks were negative. 2) As to the herons captured in Okayama City, twenty out of thirtytwo adult night herons and seven out of seventy white herons showed positive HI reaction in 1966 around the time when JE was prevalent in Okayama Prefecture. And six out of eleven night herons and one out of seven white herons showing positive HI reaction, responded positively to 2-ME sensitivity test. 3) The results indicate that white herons can be also infection source ofJE though less than in the case of night herons. 4) In the domestic fowls (white leghorn) kept at Takahashi District, eight out of twenty-seven fowls showed positive HI reaction. And six out of seven domestic fowls showing positive HI reaction responded positively to 2-ME sensitive reaction. 5) Transformation of JE antibody in the serum of hen from IgM to IgG was recognized. 6) Domestic chicken's sera having 1 : 640 of HI titer in the original serum and 1 : 320 of HI titer after 2-ME treatment were fractionated by gel filtration on Sephadex G-200 and the antibody activities present in the various fractions were determined. HI antibody activities occurred in both IgM and IgG classes of immunoglobulins. 7) Maternal HI antibodies reacting with JE virus were found in newly hatched domestic chickens from the eggs laid by hens with natural infection ofJE. And half life of HI antibodies in chicks was four days. 8) HI antibodies of JE in the serum of maternal immune-hens and chicken having maternal antibody were located in

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r-globulin fraction by starch block electrophoresis. 9) The results from 4) to 8) indicate the presence of natural infection ofJE in the domestic fowls. And domestic fowls can be infection source ofJE.

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# INFECTION OF HERONS AND DOMESTIC FOWLS WITH JAPANESE ENCEPHALITIS VIRUS WITH SPECIFIC REFERENCE TO MATERNAL ANTIBODY OF HEN (EPIDEMIOLOGICAL STUDY ON JAPANESE ENCEPHALITIS 26)

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Since KOBAYASHI *et al.* (1), KITAOKA *et at.* (2, 3), HAMMON *et al.* (4) described that a wild bird is one of the amplifire of Japanese encephalitis (JE for short), the birds were thought to play an important role in the infection of Japanese encephalitis. Namely, KOBAYASHI *et al.* (1) and KITAOKA *et al.* (3) recognized induction of viremia after inoculation of JE virus to night heron. HAMMON *et al.* (4) investigated the neutralizing antibody (NT for short) among 1705 wild birds belong 154 kinds, and they described that positive rate of NT antibody in the serum of night heron proved to be 43.1 per cent. BUESCHER and SCHERER. (5, 6) tested the hemoagglutination inhibiting antibody (HI for short) of Japanese encephalitis in the sera of herons and found the 14—49 per cent of higher positive rate of HI antibody in night heron, and 5—19 per cent of lower positive rate in white heron (5).

As to the domestic fowls, BUESCHER and SCHERER *et al.* (6) recognized the induction of viremia in chicken, after inoculation of JE virus subcutaneously over the pectral muscle. However, there is no report concerning the increase in HI antibody of JE after natural infection to chicken.

We investigated the HI antibody and also 2-mercaptoethanol (2-ME) sensitive antibody of JE in night herons (black-crowned night herons, BCNH for short, *Nycticorax nycticorax*) and white herons (plumed egrets, PE for short, *Egretta intermedia*). We also recognized the increase of HI antibody of JE in the serum of domestic fowls and examined the nature of antibody by starch block electrophoresis and Sephadex G-200 gel filtration. And we found that the maternal antibody of JE in hen is transferred from hen to chicken.

## MATERIALS AND METHODS

*Birds*:

Herons; Night herons and white herons were captured at Tsudaka Town (suburbs of Okayama City) in August of 1965 and at Okayama City in August to September of 1966, and they served as the subjects of this study.

Domestic fowls; Hens (white leghorn) were kept at Takahashi District, where Japanese encephalitis is endemic every year. Chickens were hatched from eggs laid by natural immune hens by means of artificial fertilization.

*Blood*:

Blood was taken from wing vein of herons, hens and chickens.

Procedure for measuring antibodies; The titration of HI antibody of JE was done by the method described by CLARKE and CASALS (7). The titration of 2-ME sensitive antibodies in HI reaction was done by employing the method of ANN. SCHLUEDERBERG (8).

Starch block electrophoresis; Starch blocks were prepared by the method described by KUNKEL (9). The Holt's buffer (pH 8.5, 0.045) of sodium veronal (sodium diethyl barbiturate), sodium acetate, and acetic acid was used in this experiment. The electrophoresis was conducted in starch block (1.5 cm in width, 1.0 cm in depth, 35.0 cm in length) for 27 hours in the cold room with electric current of 2.2 mA per $cm^2$. After electrophoresis, protein concentration of each section of the blocks was determined by the method of SUTHERLAND (10).

*Gel filtration with Sephadex G-200*:

The modification of the method of FLODIN and KILLANDER (11) was used. The Sephadex was washed several times with distilled water and the finely suspended particles were decanted. It was finally equilibrated with 0.1 M phosphate buffer pH 7.4. A column 82 cm in length, 19 cm in diameter was packed with Sephadex under gravity flow. A 1.5 ml sample of serum was applied to the column and elutions, made with the 0.1 M phosphate buffer, pH 7.4. The flow rate was 12 ml/hr and eluates were collected in 4 ml amounts. The protein concentration of the eluates was measured at 280 m$\mu$ by spectrophotometer (Hitachi 139, UV-Vis).

## RESULTS

*Titer of HI antibody*:

Table 1 shows titer of HI antibody in the serum of night and white herons captured in 1965 and 1966, in Okayama Prefecture. In the case of night herons captured at Tsudaka Town in 1965, two out of six adult night herons and three out of four chick's showed positive HI reaction. HI antibody of chicks may be appeared from the biting by hazardous mosquitoes, and it is not appeared by the maternal antibody, because these chicks are considered to be after thirty days old from hatching, by their body weight. Adult birds and chicks of white herons living on the top of the mountain together with night herons showed negative HI reaction. Among

Table 1 FREQUENCY DISTRIBUTION OF HI TITER OF JAPANESE ENCEPHALITIS IN THE SERUM OF BLACK-CROWNED NIGHT HERONS (BCNH), PLUMED ERGETS (PE) AND DOMESTIC FOWLS (WHITE LEGHORN)

| Date of collecting blood | August, 1965 | | | | | | September, 1965 | | | August, 1966 | | September, 1966 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Place of collecting blood | Tsudaka Town | | | | | | Oku Town | | | Takeda, Okayama City | | Takahashi City |
| Bird / Titers | BCNH | | | PE | | | BCNH | | | BCNH | PE | White leghorn |
| | Adult bird | Chicks | Total | Adult bird | Chicks | Total | Adult bird | Chicks | Total | | | hen |
| < 10 | 4 | 1 | 5 | 2 | 3 | 5 | 6 | 0 | 6 | 12 | 10 | 19 |
| 10 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 3 | 0 | 0 |
| 20 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 2 | 2 | 0 | 0 |
| 40 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 1 |
| 80 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 2 | 3 | 2 |
| 160 | 1 | 1 | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 5 | 0 | 2 |
| 320 | 1 | 1 | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 3 | 0 | 1 |
| 640 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 2 | 1 |
| 1280 | 0 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 2 | 1 | 0 |
| 2560 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 |
| Total number | 6 | 4 | 10 | 2 | 3 | 5 | 7 | 1 | 8 | 32 | 17 | 26 |
| Positive %* | 33 | 75 | 50 | 0 | 0 | 0 | 13 | 100 | 25 | 63 | 41 | 27 |
| Average antibody titer† | 240 | 586 | 448 | 0 | 0 | 0 | 20 | 20 | 20 | 387 | 405 | 211 |

* The birds with over 10 of titer in the serum are taken as positive

† Average antibody titer in the serum of birds showing positive (over 1 : 10) in HI reaction

night herons captured in Oku Town in 1965, one out of seven adult herons and one out of one chick showed positive HI reaction. Table 1 also shows HI titer of herons captured in Okayama City in 1966, twenty out of thirty-two night herons (63 % total) and seven out of seventeen white herons (41 %) showed positive HI reaction. As shown in Table 1 average HI titer of these birds captured at Tsudaka Town was 1 : 240 in the serum of adult night herons in 1965, and that of ckicks was 1 : 586. In the case of night herons captured at Oku Town in 1965, average HI titers of both adult birds and chick were 1 : 20. In the case of herons captured in Okayama City in 1966, average HI titer of adult night herons showing positive HI reaction was 1 : 387 and that of adult white herons was 405. These results indicate that night herons as well as white herons were infected by JE virus.

In this district, the rate of HI positive reaction and average HI titer both in the night and white herons showed high values, though those in the night herons showed higher value than those of white herons.

On the other hand, domestic fowls kept at Takahashi District where JE is endemic, eight out of twenty-seven birds showed positive HI reaction as shown in Table 1.

*Amout of 2-ME sensitive antibody (IgM) and transformation of antibody from IgM to IgG*:

As shown in Table 2-A, six out of eleven night herons (55 %) and one out of six white herons (17 %), showing HI positive reaction revealed posi-

Table 2-A HI REACTION OF SERUM IN THE BIRDS BEFORE AND AFTER TREATMENT OF 2-ME

| Kinds | HI titers | |
|---|---|---|
| | Original serum | After treatment of 2-ME |
| NCNH | 2560 | < 10* |
| | 1280 | < 10* |
| | 640 | < 10* |
| | 320 | < 10* |
| | 320 | 40* |
| | 160 | < 10* |
| | 640 | 640 |
| | 160 | 160 |
| | 160 | 80 |
| | 80 | 80 |
| | 80 | 80 |
| PE | 160 | 20* |
| | 1280 | 1280 |
| | 640 | 640 |
| | 640 | 640 |
| | 80 | 80 |
| | 80 | 80 |
| WL | 160 | < 10* |
| | 80 | < 10* |
| | 80 | < 10* |
| | 640 | 40* |
| | 320 | 40* |
| | 160 | 40* |
| | 40 | 20 |

* 2-ME sensitive antibody

tive 2-ME reaction. In the case of domestic fowls, the blood of which was collected in September, six out of seven birds showed positive reaction of 2-ME sensitive antibody. In the case of domestic fowls, 2-ME sensitive antibody in the serum collected in September decreased and 109 days afterward 2-ME resistant antibody increased in the serum collected in

January of next year as shown in Table 2-B.

On the other hand, twenty domestic fowls in the southern part of Okayama Prefecture showed negative HI reaction.

Table 2-B TRANSFORMATION OF ANTIBODY FROM 2-ME SENSITIVE ANTIBODY TO 2-ME RESISTANT ANTIBODY

| Kinds | HI titers | |
|---|---|---|
| | Original serum | After treatment of 2-ME |
| WL | 640** | 40* |
| | 640*** | 320 |

* 2-ME sensitive antibody
** Serum collected in September
*** Serum collected in January of the following year

*Sephadex gel filtration* :

Serum of hen used in this experiment showed HI titer of 1 : 160 and its HI titer after treatment with 2-ME was 1 : 320. As shown in Fig. 1, HI

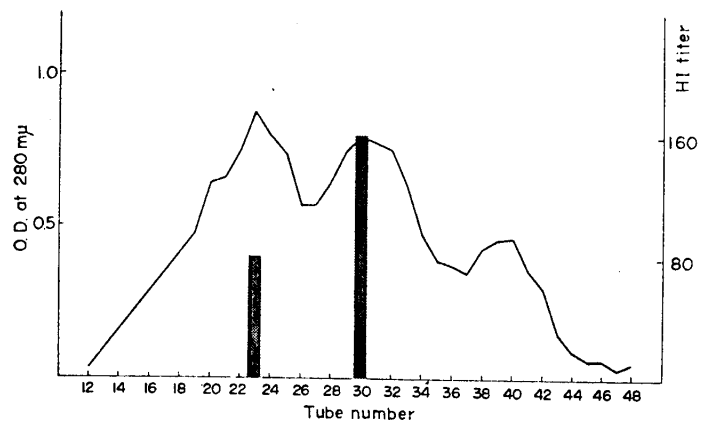


Fig. 1 Distribution of hemagglutination-inhibiting antibody in the fractions of hen's serum by Sephadex G-200 gel filtration. Curve of solid line shows protein concentration and black column hemagglutination-inhibiting titer.

antibodies appear in fraction 23 and 30 corresponded to the peak of absorption curves of IgM and IgG respectively. From this experiment, it was demonstrated that IgM and IgG of JE in the serum were found by natural infected hen.

*Disappearance of maternal HI antibody of JE virus in the serum of chickens* :

Chickens were hatched from eggs laid by naturally infected hens with JE previously treated by artificial fertilization with samen from the cock without infection of JE. The HI titer of hen used in this experiment was

1 : 640. The sera were taken at appropreate interval after hatching. Fig. 2 shows HI antibody titer of chick's serum plotted against days after its hatching and HI titer just after hatching is calculated as 1 : 600. And date when maternal antibody disappeared is calculated as the 38th day by extrapolation method by the line as shown in Fig. 2. The relationship between days after hatching and logarithm of HI titer of chicken show lineal. This line is similar to the line showing disappearance of serum protein in mammalians. Half life of maternal antibody in the serum of chick was calculated from rate of disappearence as four days, and it is almost the same as that (12) of 3.2 days of half life of serum $\gamma$-globulin determined by glycine -$N^{15}$.

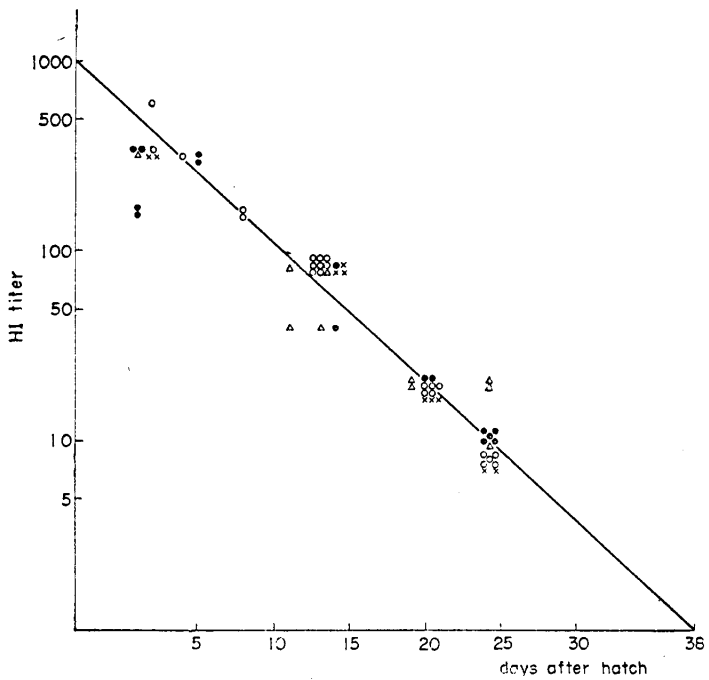


Fig. 2 Rate of disappearance of maternal hemagglutin-ation inhibiting antibodies in the sera of chickens hatched from eggs laid by hen naturally infected with Japanese encephalitis. Chicken hatched on 1st April→●, 11st April→△, 2nd May→○, 1st June→×.

*Starch block electrophoresis*:

Starch block electrophoresis of hen's serum having 640 of HI titer and also chicks serum having 320 of HI titer was conducted. As shown in Fig. 3 and Fig. 4, the HI antibodies of hen and chicken were contained in $\gamma$-globulin fraction respectively.

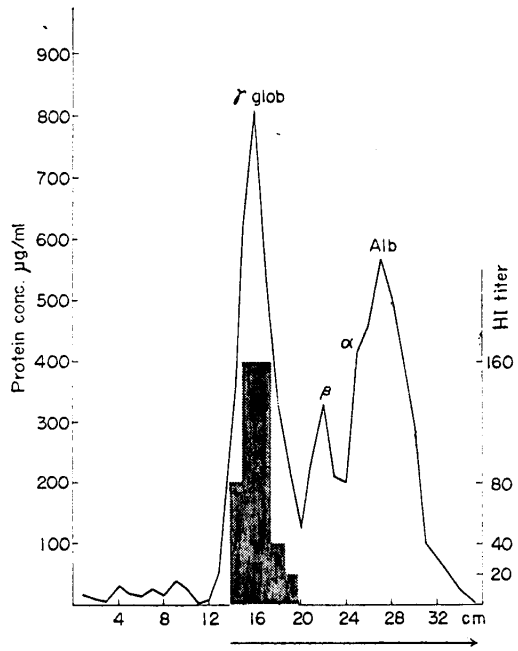


Fig. 3 Distribution of hemagglutination-inhibiting antibody in the serum of hen naturally infected with Japanese encephalitis by starch block electrophoresis. Curve of solid line shows protein concentration and black column hemagglutination-inhibiting antibody titer.

## DISCUSSION

*HI reaction* :

BUESCHER and SCHERER (5, 6) have described that herons and swines play an important role in infection of JE virus as amplifier. And herons have specific significance compared with swines as follows.

(1) Herons transfer JE virus from their nests to wild animals and person at any place where they can fly at the time when they are in the state of viremia.

(2) Herons may transfer JE virus which may live out through the winter in the southern part of Japan to the northern regions as migratory bird.

(3) In the countries like China, Formosa, the Philippines and Java, JE is prevalent at the time when it is winter in Japan. Herons from these countries migrate to Japan in the beginning of summer and may transport JE virus with them.

Swines living in Okayama Prefecture showed 100 per cent of positive

HI reaction during the periods of 1965 and 1966. On the other hand, night herons showed 60 per cent positive HI reaction at most suggesting that the night herons show lesser potential of infection to person than swine. As to the white herons, 42 per cent positive HI reaction was found in Okayama Prefecture in 1966, proving that they play a partial role as amplifier.

*2-ME sensitive antibody*:

2-ME sensitive antibody has been proven to be present in the sera of birds in this experiment just as in the sera of mammalians. The transformation of JE-antibody in hen's serum from IgM to IgG has also been recognized. Therefore, early stage of infection of bird can be estimated by the use of 2-ME sensitive reaction. Namely, these results show that 1) 55 per cent of positive night herons showing positive HI reaction and 20 per cent positive white herons showing positive HI reaction are 2-ME sensitive. 2) In the case of domestic fowls, it was found that 2-ME sensitive antibodies found in September of 1966 decreased and 2-ME resistant antibody increased in January of 1967. This indicates that domestic fowls suffered from natural infection through hazardous mosquitoes are produced IgM and thereafter IgG. And it is considered that domestic fowls can be one of the sources of JE virus infection because experimental vireuria was recognized by inoculation of JE virus to domestic fowls by KITAOKA (3) and by BUESCHER and SCHERER (5).

*Gel filtration with Sephadex G-200*:

The presence of 2-ME sensitive and resistant antibodies in the form of IgM and IgG is confirmed by the presence of HI antibodies in both 19S (IgM) and 7S (IgG) peaks by Sephadex G-200 gel filtration.

*Starch block electrophoresis*:

As HI reaction was found in the $\gamma$-globulin fraction in the serum of hen and chicks by starch block electrophoresis, it was confirmed that HI antibody of JE was produced in the $\gamma$-globulin fraction of hen after natural infection and it was transfered to chicks throught egg yolk.

*Transmisson of maternal antibodies*:

Transmisson of maternal antibodies from hens infected with S. Pullorum to chickens through yolk of eggs was recognized by WATANABE (13). And BUESCHER and SCHERER (6) reported that maternal HI and neutralizing antibodies of JE in the serum of BCNH by experimental and natural infection were transmitted to chicks. However, there is no report telling maternal antibody of domestic hen produced by natural infection can be transmitted to chickens, and it decreases with lapse of the time after hatching. These results indicate that half-life of maternal antibody

in chicken is only 3 days and it disappear 38 days after hatching. This also indicates that vaccination of JE to chicken should be done 38 days after hatching.

## CONCLUSION

In order to ascertain whether black-crowned night herons (BCNH), white heron (Plumed Egrets (PE)) and domestic fowls are infected by JE virus and they serve as infection source of JE, hemoagglutination inhibiting antibody and its 2-ME sensitive antibody in the sera of these birds were determined. Physico-chemical nature of fowl's antibody of JE produced by natural infection and their maternal antibody in the sera of chicks were examined.

The results are briefly summarized as follows.

1) As to the herons captured in Tsudaka Town, two out of six adult night herons and three out of the four chicks showed positive HI reaction. On the other hand, HI reaction in the sera of two adult white herons and three chicks were negative.

2) As to the herons captured in Okayama City, twenty out of thirty-two adult night herons and seven out of seventy white herons showed positive HI reaction in 1966 around the time when JE was prevalent in Okayama Prefecture. And six out of eleven night herons and one out of seven white herons showing positive HI reaction, responded positively to 2-ME sensitivity test.

3) The results indicate that white herons can be also infection source of JE though less than in the case of night herons.

4) In the domestic fowls (white leghorn) kept at Takahashi District, eight out of twenty-seven fowls showed positive HI reaction. And six out of seven domestic fowls showing positive HI reaction responded positively to 2-ME sensitive reaction.

5) Transformation of JE antibody in the serum of hen from IgM to IgG was recognized.

6) Domestic chicken's sera having 1 : 640 of HI titer in the original serum and 1 : 320 of HI titer after 2-ME treatment were fractionated by gel filtration on Sephadex G-200 and the antibody activities present in the various fractions were determined. HI antibody activities occurred in both IgM and IgG classes of immunoglobulins.

7) Maternal HI antibodies reacting with JE virus were found in newly hatched domestic chickens from the eggs laid by hens with natural infection of JE. And half life of HI antibodies in chicks was four days.

8) HI antibodies of JE in the serum of maternal immune-hens and chicken having maternal antibody were located in $\gamma$-globulin fraction by starch block electrophoresis.

9) The results from 4) to 8) indicate the presence of natural infection of JE in the domestic fowls. And domestic fowls can be infection source of JE.

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