

A SEROLOGICAL SURVEY ON HEMAGGLUTINATING ENCEPHALOMYELITIS VIRUS INFECTION IN TAIWAN

H. J. Tsai,¹ D. F. Lin,¹ Y. S. Lu¹
K. S. Tsai,² Y. L. Lee,¹ C. Lee¹ and S. T. Huang¹

Taiwan Provincial Research Institute for Animal Health.

A total of 1452 sera of sows, more than 12-month-old of age, were collected from 70 farms over 4 geographical regions in Taiwan. Hemagglutinating-Inhibition (HI) test for detection of antibody were carried out on microtitre system by using hemagglutinating encephalomyelitis virus (HEV) as antigen at dose of 4 hemagglutination (HA) units. Result of the tests revealed that sera from 65 out of 70 farms were shown positive reaction against HEV antigen, the average positive rate was 28.65% (416/1452).

The distribution of the positive reaction revealed that the southern region was the highest (40.75%); and that in eastern region was the lowest (14.17%); northern and middle regions were 27 and 22.4% respectively.

Antibodies against HEV were proved from sera collected in Taiwan in 1979 and every subsequent year. Sera from 1979, 45.32% (155/342) of sera collected over 6 counties in southern region of Taiwan were antibody positive, therefore, it is concluded that HEV infection may be widespread in Taiwan at least since 1979.

Hemagglutinating encephalomyelitis virus (HEV), classified as a coronavirus,^(9,16) causes encephalomyelitis or so-called vomiting and wasting disease in suckling piglets. In 1958, the disease in suckling piglets. In Canada,⁽¹⁹⁾ since then the disease was reported in England,^(1,2,3,11) Belgium,⁽¹⁸⁾ France,⁽¹²⁾ Switzerland,⁽²⁰⁾ Germany⁽⁸⁾ and Australia.⁽⁶⁾

In an immunological survey conducted on 116 adult pigs in Canada prior to 1964, 111 pigs (96%) had HI antibodies against HEV.⁽⁷⁾ On hog farm surveyed in England over a period from 1960 to 1969, the positive rate varied from 10.6 to 82.5.⁽²⁾ In a survey performed in the United States in 1971, positive rate was 76%.⁽¹⁴⁾ Even in Northern Ireland and Japan where the virus had never been isolated before, 50%⁽¹³⁾ and 49.0%⁽¹⁰⁾ of the porcine sera examined were known to possess antibodies against HEV.

In Taiwan, no clinical disease have been reported also, but a serological survey was carried out on 747 porcine sera collected from pigs of Taiwan Sugar Corporation in 1977 to 1978, it was found 308 (41.2%) among them had the antibody. (4) In order to understand the real epidemiological situation, an island-wide antibody survey and a retrospective studies were carried out.

1. Sera Collection:

A total of 1452 sera of sows (12 months of age) were collected randomly from 70 pig farms of 4 regions in Taiwan in 1983. Another 541 sera collected before 1983. were selected for a retrospective study, among them 199 sera were collected in Yunlin County during a period from 1980 to 1982. Other 342 sera were collected over 6 counties (Changhwa, Yunlin, Chia-I, Tainan, Kaoshiung and Pintung.) in 1979.

All the collected sera were stored at -20°C until tested.

2. Removal of nonspecific inhibitors in sera:

In order to remove non-specific inhibition factors in hemagglutinations, 0.2ml sera were treated with 0.4ml 10% chicken packed erythrocytes in 4°C overnight, then treated with 0.5ml 25% kaolin solution for 30 minutes in room temperature. All the sera were then inactivated by 56°C , 30 minutes treatment.

3. Antigen:

HEV (supplied by National Institute of Animal Health, Japan) propagated in primary swine kidney cell culture was used as the antigen.

4. Hemagglutination (HA) and Hemagglutination Inhibition (HI) Tests:

All HA test and HI test were carried out in microtitre system.

HA test: The HEV culture fluid was diluted in 0.025ml amounts in 2-fold steps starting at 1:2. The diluent was 0.85% saline. To each dilution 0.025ml of 1% chicken erythrocyte suspension was added. The mixtures were shaken by mechanical shaker, then allowed to stand for 45 minutes before examined for settling pattern of the cells. One HA

unit was determined in the highest dilution showing a distinct pattern of agglutination, with no central "button" of settle cells.

HI test: For the HI test, serum was diluted in 0.025ml amounts of saline by the 2-fold step starting at 1:10. To each serum solution was added 0.025ml of antigen containing 4 HA units. the contents were mixed and held at room temperature for 60 minutes. Then 0.025ml of 1% suspension of chicken erythrocytes was added to each well. The tests were read after a further incubation for 1 hour. The serum titer was expressed as the reciprocal of the highest dilution of serum that showed complete inhibition of viral hemagglutination. Titers of 1:40 and higher were regarded as positive according to the Mengling,⁽¹⁴⁾ Girard et al.⁽⁷⁾ and Hirai et al.⁽¹⁰⁾

RESULTS

A total of 1452 porcine sera were collected randomly from 70 pig farms of 4 geographical regions in Taiwan in 1983. The results of HI antibody against HEV are summarized in Table 1-3. The positive rate was much higher in southern region (40.75%) than in other regions. Sera from all 26 farms in southern region had HEV antibody and one of them had positive rate as high as 95%. On the other hand, it was much lower in eastern region (14.17%). The positive rate of northern and middle regions were 27 and 22.4% respectively. Collectively, 65 farms were positive in HEV antibody, among them 26 farms (37.14%) had positive rate under 20%, only one farm had positive rate higher than 80%. The average positive rate in Taiwan was 28.65%(416/1452).

By the retrospective study, HI antibodies against HEV were proved in this country in 1979 and confirmed in every subsequent year. Porcine sera were collected in the field over a period from 1979 to 1982 in Yunlin County and tested for HI antibodies. The result obtained was shown in Table 4. Since the number of serum sample collected was limited, the positive rates were rather scattered.

Further examination of the 342 sera which were collected over 6 counties of southern Taiwan in 1979. The result obtained was shown in Table 5. The average positive rate of this region (45.3%) was closed

Table 1. Hemagglutinating Encephalomyelitis Antibody Survey in 4 Geographical Regions of Taiwan in 1983

| Regions | No. of Farm Survey | No. of Positive Farm | No. of Sera Tested | No. of Positive Sera | Positive Rate % |
|---------|--------------------|----------------------|--------------------|----------------------|-----------------|
| North | 15 | 12 | 300 | 81 | 27.0 |
| Middle | 23 | 22 | 375 | 84 | 22.4 |
| South | 26 | 26 | 530 | 216 | 40.75 |
| East | 6 | 5 | 247 | 35 | 14.17 |
| Total | 70 | 65 | 1452 | 416 | 28.65 |

Table 2. The Distribution Range of HEV Antibody Positive Farm in Taiwan in 1983

| Regions | No. of Antibody Positive Farm Range of Positive Rate. % | | | | | |
|---------|---|-------|-------|-------|-------|--------|
| | 0 | 1-20 | 21-40 | 41-60 | 61-80 | 81-100 |
| North | 3 | 4 | 4 | 3 | 1 | 0 |
| Middle | 1 | 11 | 10 | 1 | 0 | 0 |
| South | 0 | 7 | 9 | 5 | 4 | 1* |
| East | 1 | 4 | 1 | 0 | 0 | 0 |
| Total | 5 | 26 | 24 | 9 | 5 | 1 |
| (%) | 7.14 | 37.14 | 34.29 | 12.86 | 7.14 | 1.43 |

* This farm had 95% positive rate.

Table 3. Distribution of HI Antibodies Titre Against HEV in Number of Pigs in Taiwan in 1983

| Regions | No. of Distribution of HI Titre | | | | | | | |
|---------|---------------------------------|------|-------|-------|------|-------|-------|-------|
| | <1:10 | 1:10 | 1:20 | 1:40 | 1:80 | 1:160 | 1:320 | 1:640 |
| North | 55 | 91 | 73 | 47 | 17 | 15 | 2 | 0 |
| Middle | 158 | 38 | 95 | 58 | 20 | 3 | 3 | 0 |
| South | 122 | 78 | 114 | 125 | 65 | 21 | 4 | 1* |
| East | 116 | 50 | 46 | 22 | 9 | 1 | 3 | 0 |
| Total | 451 | 257 | 328 | 252 | 111 | 40 | 12 | 1 |
| (%) | 31.06 | 17.7 | 22.59 | 17.36 | 7.64 | 2.75 | 0.83 | 0.07 |

* Titre of 1:40 or higher are considered as positive.

Table 4. Distribution of HI Antibodies against HEV in Yunlin County by Calender Year

| Year | No. of Farm Survey | No. of Sera Tested | No. of Positive Sera | Positive Rate. % |
|------|--------------------|--------------------|----------------------|------------------|
| 1979 | 2 | 60 | 36 | 60.0 |
| 1980 | 2 | 70 | 32 | 45.71 |
| 1981 | 2 | 76 | 39 | 51.32 |
| 1982 | 2 | 53 | 40 | 75.47 |

*All the farm under survey were antibody positive farm.

Table 5. Retrospective Survey on Antibodies against HEV by Porcine Sera which were Collected over 6 Counties in Southern Taiwan in 1979

| Counties | No. of Farm Survey | No. of Sera Tested | No. of Positive Sera | Positive Rate. % |
|----------|--------------------|--------------------|----------------------|------------------|
| Changhwa | 1 | 39 | 26 | 66.67 |
| Yunlin | 2 | 60 | 36 | 60.00 |
| Chia-I | 1 | 30 | 13 | 43.33 |
| Tainan | 6 | 119 | 36 | 30.25 |
| Kaoshing | 2 | 50 | 20 | 40.00 |
| Pintung | 1 | 44 | 24 | 54.55 |
| Total | 13 | 342 | 155 | 45.32 |

*Only one farm in Tainan County was antibody negative farm.

to the result obtained in 1983 (40.75%).

DISCUSSION

In the present serological survey, more than 90% of pig farms investigated were HEV antibody positive farm, approximately 30% of the sera tested were positive for antibodies against HEV. Therefore, that HEV infection may be widespread in Taiwan.

Although no outbreaks or isolation of HEV virus had been reported in Taiwan yet, inapparent infection may present as reported in many

countries.^(10,15) In Northern Ireland, McFerran et al.⁽¹³⁾ demonstrated that HEV may have been introduced between 1960 and 1967 without the development of obvious clinical disease. A strain of HEV isolated in the USA was known to be capable of the producing the disease experimentally in susceptible young pigs.^(5,15) It is considered to be of low virulence than strains isolated in Canada and England. Likewise, it may be the strain of low virulence cause the inapparent infection in Taiwan.

In the present survey, HI antibodies against HEV were proved in 1979, there-after they were confirmed in every subsequent year. In 1979, the average positive rate of sera collected over 6 counties in southern region of Taiwan was closed to the result in 1983. Because of its highest positive rate in 1983, it was suspected that the HEV infection was first introduced into Taiwan in southern region. But, the more evidence was needed to support this speculation. Nevertheless it could be concluded that might be already widespread in Taiwan before 1979.

In the present study, except the titre of 1:10, the percentage of the titre were decreased as the titre rised. Nearly half of the sera (48.67%) had the titre of 1:10 or lower.

No sera had the titre higher than 1:640. This result is in accordance with the report of Chang⁽⁴⁾ and Mengeling,⁽¹⁷⁾ but had great difference to Hirai's report.⁽¹⁰⁾ In Chang's report, the sera had the titre higher than 1:512 only occupied 2% of the total sera tested. Mengeling examined 349 sera in Iowa, USA, among them 106 (30.37%) had the HI titre 1:10 or lower, none of them had the titre higher than 1:640. But in Hirai's report, HI antibody against HEV were strongly distribution in 1:10 (49%) and 1:640 (39%). The reason of this difference is unknown, more information is needed to understand its cause.

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台灣豬血球凝集性腦脊髓炎病毒之抗體調查

蔡向榮¹ 林地發¹ 呂榮修¹ 蔡貴雄²
李永林¹ 李全¹ 黃士則¹

台灣省家畜衛生試驗所

以血球凝集抑制反應調查本省母豬血球凝集性腦脊髓炎病毒之抗體，結果發現受檢之 70 個養豬場中只有 5 場為抗體陰性，陽性率在 20% 以下者有 26 場 (37.14%)，陽性率在 60% 以上者有 6 場 (8.57%)，其中有一場高達 95%。在全部受檢之 1452 頭豬血清中有 416 頭為陽性，平均抗體陽性率為 28.65%。

以地區而言，南部地區抗體陽性率最高 (40.75%) 且所有 26 個受檢豬場皆為抗體陽性，東部地區之抗體陽性率則為最低 (14.17%)，北部及中部地區分別為 27 及 22.4%。

追蹤自 1979 年起之本省豬血球凝集性腦脊髓炎之抗體，調查結果發現每年均有陽性抗體存在。1979 年由彰化、雲林、嘉義、台南、高雄及屏東 6 縣市所收集之 342 頭豬血清中發現 155 頭抗體陽性血清 (45.32%)。顯示台灣至少從 1979 年起即已可能有豬血球凝集性腦脊髓炎之廣泛性潛伏感染。

