A serological survey of the prevalence of Aujeszky's disease antibodies in Thailand using enzyme-linked immunosorbent assays (ELISA), serum neutralization (SN) and latex agglutination tests (LT)

INTRODUCTION

Aujeszky's disease (AD) causes severe economic losses in the swine industry worldwide. In Thailand the first reported outbreak of the disease was in Nakhonpathom province, an area of major pig production (12). A further five outbreaks occurring between 1979 and 1980 were reported by SUNYASOOTCHAREE et al. (13), and since 1982, there have been several outbreaks of AD in six of the southern provinces of Thailand (1).

Until now, studies on AD in Thailand were performed on an isolated basis, and only a few provinces participated in each study. Also, most of these studies were based on virus isolation.

The present report summarizes the results of a serological survey of AD based on 800 samples taken from 86 swine herds in 26 provinces of Thailand. The tests used were "Enzygnost-Aujeszky" ELISA (Behring, Germany), serum neutralization test (SNT), and "Aujeszky-latex kit" (LT) (Iffa Mérieux, France). This is the first study to take place in Thailand using ELISA-alkaline-phosphatase, serum neutralization and LT methods to demonstrate the extension of the AD virus antibodies and its presence in swine herds. The applied techniques of these three tests have been described elsewhere (5).

MATERIALS AND METHODS

Collection of samples

Between February and April 1988 samples were collected from swine herds in 26 provinces of Thailand (map 1). These samples were obtained on a voluntary basis from each of the farm owners' swine herds by officers of the Department of Livestock Development (DLD). None of the pigs sampled was displaying visible signs of AD. The samples (from the jugular vein) were randomly taken from pigs without regarding the age (i.e. from piglets up to pigs more than eight years old), sex and breed (Duroc, Large-white, Landrace, hybrid...). In addition to the normal serum extracts, blood from each of the samples was blotted onto a special paper (0.025 ml Microdiluter Delivery Tester, MDT, Dynatech, Switzerland). The MDT-paper was cut into 4.0 x 4.6 cm' strips permitting the blood samples to be placed in a slide holder to avoid contamination and for ease of carriage. Each strip was given an identifier to associate it with a particular pig. After the blood was blotted onto the MDT-paper strips, these were allowed to dry in the slide holder, and then stored in plastic bags at room temperature until investigation.

Sero logical tests

Detection of the antibodies for the AD virus was carried out by means of the ELISA, SNT and LT methods.

ELISA

A total of 800 serum samples and 800 corresponding blood eluates were tested using ELISA. Each of the MDT "blood paper" strips was punched in 2 discs measuring 5.5 mm in diameter containing each 0.02 ml blood. These were then soaked in 1.056 ml dilu-
tion buffer of "Enzygnost-Aujeszky". After incubation at
37 °C for 20 min, the resulting solution was further diluted
with the dilution buffer to reach a blood concentration of
1:88 (assuming a haematocrit value of about 40%). The
serum samples were diluted 1:44. Eluates and sera
were tested for antibodies against the AD-virus using the
Behring ELISA procedure.

SNT
The procedure used was adapted with some minor modi-
fications from a similar method used in the diagnostic
center in Oberschleissheim, Bavaria, FRG. It was carried
out using flat-bottomed 96-well microtiter plates (Falcon
3070 ; Becton Dickinson & CO., USA) with pairs of
samples of heat-inactivated (56 °C, 30 min) sera diluted
1:1, 1:2, 1:4 and 1:8, a virus dose 50 of
Kid50/0.025 ml and foetal calf lung cells. The medium
used for cell propagation was Eagle's minimum essential
medium (E MEM) pH 7.2-7.4, consisting of Earle's salt,
glutamine, 20 mM hepes and 0.85 g/l NaHCO3 (Flow
laboratories, Germany). To this were added 5% or 2%
foetal calf serum, 5% lactalbumin-hydrolysate solution
and 0.2% gentamycin solution (50 μg/ml).

Only serum samples were tested by SNT. A total of 640
samples which had already been tested using ELISA and
gave either negative or weakly positive results, or which
gave conflicting results using ELISA between serum
samples and corresponding blood eluate were tested
using the SNT procedure.

LT
A total of 59 serum samples and 123 blood eluates
were evaluated using LT. These were:
- suspicious samples which gave different results with
ELISA (blood eluate and/or serum) and SNT (serum
only);
- those with positive results with ELISA for both blood
eluates and sera;
- those which had negative results using both ELISA and
SNT.

Four paper discs from each blood sample were eluted
with 240 μl of "Enzygnost-Aujeszky" diluent in an incuba-
tor at 37 °C for 20 min. Assuming as before a haematocrit
value of about 40%, this yielded blood eluates with a
serum dilution of 1:5. Eluates and corresponding sera
(undiluted) were tested using the Iffa Merieux LT proce-
dure. Agglutination time for each serum sample was
about six minutes and that for blood eluates about 13
minutes. Reliable results were only obtained after these
periods had elapsed.

RESULTS

ELISA
A total of 210 (26.3%) of the 800 serum samples and 142
(17.8%) of the corresponding blood eluate samples were
positive when tested by means of ELISA (table 1).

The higher percentage of positive results for serum
camps was due to the greater sensitivity of the assay to
serum samples rather than to blood eluates, because
background optical density (OD) levels were always
TABLE I  Seroreactivity as determined by ELISA ('Enzygnost-Aujeszky', Behring) when 800 serum samples and 800 corresponding blood eluate samples were tested.

<table>
<thead>
<tr>
<th>Sample type</th>
<th>Total</th>
<th>Positive results</th>
<th>Negative results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum</td>
<td>800</td>
<td>210 (26.25 %)</td>
<td>590 (73.75 %)</td>
</tr>
<tr>
<td>Blood eluate</td>
<td>800</td>
<td>142 (17.75 %)</td>
<td>658 (82.25 %)</td>
</tr>
</tbody>
</table>

higher than those of the serum samples when using a positive-negative cut-off point of 0.2 OD as recommended in the Behring method.

SNT
Most of the samples tested using SNT were those that gave negative results with ELISA. Also evaluated using SNT were those samples which, when tested using ELISA, yielded either a weakly positive result (0.4 > OD > 0.2), or gave conflicting results between sera and the corresponding blood eluates. The results indicated that 143 out of 640 serum samples (22 %) were positive (table II).

TABLE II Seroreactivity as determined by SNT when 640 serum samples chosen because they gave negative, suspicious, or weakly positive results (by ELISA) were tested.

<table>
<thead>
<tr>
<th>Test</th>
<th>Total</th>
<th>Positive results</th>
<th>Negative results</th>
</tr>
</thead>
<tbody>
<tr>
<td>SNT</td>
<td>640</td>
<td>143 (22.3 %)</td>
<td>497 (77.7 %)</td>
</tr>
</tbody>
</table>

LT
Most of the 182 samples (60 serum samples and 123 blood eluates) tested using LT gave suspicious test results in ELISA (blood eluate-serum) and/or SNT (serum only). The samples which gave negative results using SNT and ELISA and positive results using ELISA alone were tested and yielded also negative and positive results, respectively, using LT. Out of these 182 samples, 63 were clearly positive (table III).

TABLE III Seroreactivity as determined by 'Aujeszky-Latex-Kit' (Iffa-Mérieux) when 59 serum samples and 123 blood eluate samples were tested.

<table>
<thead>
<tr>
<th>Sample type</th>
<th>Total</th>
<th>Positive results</th>
<th>Negative results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum</td>
<td>59</td>
<td>10 (16.9 %)</td>
<td>49 (83.1 %)</td>
</tr>
<tr>
<td>Blood eluate</td>
<td>123</td>
<td>53 (43.1 %)</td>
<td>70 (56.9 %)</td>
</tr>
<tr>
<td>Total</td>
<td>182</td>
<td>63 (34.6 %)</td>
<td>119 (65.4 %)</td>
</tr>
</tbody>
</table>

TABLE IV  Seroreactivity according to the age as determined by ELISA ('Enzygnost-Aujeszky') when 800 serum samples and 800 corresponding blood eluate samples were tested.

<table>
<thead>
<tr>
<th>Age of pigs (months)</th>
<th>ELISA serum samples</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Positive</td>
</tr>
<tr>
<td>0-1</td>
<td>3 (1.43 %)</td>
</tr>
<tr>
<td>1-3</td>
<td>31 (14.76 %)</td>
</tr>
<tr>
<td>3-8</td>
<td>46 (21.90 %)</td>
</tr>
<tr>
<td>&gt; 8</td>
<td>130 (61.90 %)</td>
</tr>
<tr>
<td>Total</td>
<td>210</td>
</tr>
</tbody>
</table>

$\chi^2 = 8; P < 0.05$.

On the basis of ELISA results, an increasing proportion of the samples were positive with increasing age of the pig, ranging from < 1.5 % for piglets less than one-month old up to almost 62 % for pigs over eight months old (table IV).

From calculation of the chi-square values ($X^2 = 8; p < 0.05$) it was apparent that the number of positive test results increased with increasing age of pigs.

In addition, there were significant dependencies between the age of the pigs and results of the remaining tests, except for serum samples using LT (results not shown).

DISCUSSION

A total of 800 samples were collected randomly from pigs in 86 swine herds in 26 provinces of Thailand. These samples and a corresponding number of blood eluates were initially tested using "Enzygnost-Aujeszky" (ELISA, Behring, Germany). Positive results were obtained for 210 swine serum samples (26 %) and 142 of the corresponding blood eluates (18 %).

Next, from these 800 samples, 649 were tested using the microtiter-SNT and a subsequent 182 were then investigated with "Aujeszky-Latex kit" (LT, Iffa Mérieux, France). SNT detected AD virus antibodies more successfully than the ELISA test.

Using LT, the samples yielded positive or negative results respectively if they had been positive under ELISA (serum and blood eluate), or negative under both ELISA (serum and blood eluate) and SNT, as described earlier.
To avoid a possible incorrect positive or negative diagnosis, the test results for each sample, both for blood eluate and for serum, were then reconsidered.

Since the results from ELISA showed that the test for blood eluates had a lower sensitivity than that for the corresponding serum, samples were judged positive when at least one of the following tests gave a positive result: ELISA (serum), LT (serum), LT (blood eluate) and SNT (for dilution > 1 : 2). On this basis, a farm with a swine herd from which just one pig gave positive results was deemed positive.

Although there were control serum samples at each dilution (1 : 1, 1 : 2, etc.) using SNT, samples which gave a positive result at the dilution of 1 : 1 were not immediately judged as positive. If the SNT results at the dilution of 1 : 1 were positive (in contrast to the other test results for the same sample), the samples from a positive and from a negative herd were then deemed suspicious and negative, respectively.

After this examination of the results, 44 out of 800 samples from swine herds in three of the 26 provinces (Nakhonratchasima, Khon-Kaen and Ayutthaya) were negative. Positive test results were found in the remaining provinces (see map).

Taking into consideration case histories of disease outbreak, the pattern of vaccination, and the origin of the pigs in these farms (this information came from questionnaires filled out by the farm owners), not all of the positive samples were due to natural infection.

In some farms, pigs were vaccinated two years earlier when they were six months old with an inactivated vaccine or with an AD vaccine whose name and type was not indicated. Not all farmers were able to supply precise information about their livestock; although many did not directly use vaccine, they had purchased pigs from other farms and were unsure whether these had already been vaccinated.

It was therefore not certain whether some of the seropositive results were due to natural infection with AD or due to the effect of vaccination against AD. However, it is likely that most of these positive test results were due to the consequence effect of the spread of the disease. Firstly, while in the case of suckling pigs and piglets AD antibodies could normally persist for about six to nine weeks (2, 10), if the chi² values were taken into account, it became apparent that the older the pigs, the greater the probability that the results of the tests would be positive. Also, less than 30 positive samples were from piglets under three months old. This means that the remaining number of positive results in the older animals could not have been due to the effects of maternal immunity.

Secondly, although there were limitations to the accuracy of the information returned in the questionnaires filled out by the farmers, one important fact was clear, that less than 50% of the pigs bought by small farm owners originated from large farms. The balance had thus been reached through trading between small holders who, in contrast to the owners of big farms, generally do not vaccinate their herds because of the cost of this operation. There is therefore a high probability that the remaining pigs from which positive samples had been taken were in fact positive due to natural infection.

To date, several studies have been published on AD in Thailand, but the scope of these has been limited to clinical and/or virus diagnosis (4, 8, 9, 12, 13, 14). Other studies described the methods by which antibodies for the AD virus might be detected (3, 11), but the number of positive results from the tests described in these two reports was not shown, or in no case were these tests performed using ELISA, SNT and LT. In addition, the history of AD vaccination was not discussed.

In the past, when AD virus antibodies were detected, it was not possible to differentiate between vaccinated and infected pigs. However, there are now recombinant vaccines which do not produce antibodies to glycoprotein I (gI), in contrast to natural infection with field strain virus (6, 7). As a result, the AD virus antibodies in serum samples from animals vaccinated using a recombinant vaccine differ from those unvaccinated and infected animals. Until now, recombinant vaccines have not been used in Thailand.

In conclusion, we believe that most of the positive test results found in our investigations were the consequence of the natural infection. Therefore we conclude that Aujeszky’s disease is widespread in Thailand.

ACKNOWLEDGEMENTS

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The presence of Aujeszky’s disease (AD) antibodies in eluates of whole blood on filter paper and corresponding sera from Thai pigs was determined by ELISA, SNT and LT. From a total of 800 samples tested by ELISA, 26 % of the sera and 18 % of the eluates showed positive results. From 640 samples tested by SNT and chosen because they gave negative, suspicious, or weakly positive results by ELISA, 22 % were positive. A total of 182 suspicious samples were also tested by LT, and among them 63 (35 %) were clearly positive. The investigation demonstrated that the older the animal, the greater the probability that antibodies would be found. Owner surveys tended to state that few animals had been vaccinated. This coupled with the high frequency of antibodies detected, indicates that AD-infection among Thailand’s swine population is a common problem. Key words : Pig - Aujeszky’s disease - ELISA - Immunological test - Thailand.

REFERENCES


