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

LEAMCHARASKUL (P.), RENNER-MÜLLER (I.C.E.), REIMANN (M.), MUNZ (E.). Enquête sérologique sur la prévalence des anticorps de la maladie d'Aujeszky en Thaïlande à l'aide de la méthode ELISA et des tests de séroneutralisation (SN) et d'agglutination de latex (LT). *Revue Élev. Méd. vét. Pays trop.*, 1991, 44 (2) : 135-140

La présence des anticorps de la maladie d'Aujeszky dans les éluats de sang complet sur filtre de papier chez les porcs thaïlandais a été déterminée par les tests ELISA, SN et LT. Sur un total de 800 échantillons testés par la méthode ELISA, 26 p. 100 des sérums et 18 p. 100 des éluats ont donné des résultats positifs. Sur 640 prélèvements soumis au test de la séroneutralisation, parce qu'ils avaient montré des réponses négatives, douteuses ou faiblement positives au test ELISA, 22 p. 100 se sont révélés positifs. Enfin, un total de 182 prélèvements suspects ont également été testés par agglutination au latex. Parmi eux, 63 ont été nettement positifs (35 p. 100). L'enquête révèle que plus l'animal est vieux, plus grande est la probabilité de trouver des anticorps. Les interrogatoires des propriétaires tendent également à démontrer que peu d'animaux avaient été vaccinés. Ces constatations, associées à la grande fréquence des anticorps détectés, indiquent que la maladie d'Aujeszky chez les porcs thaïlandais est un problème courant. *Mots clés* : Porc - Maladie d'Aujeszky - Test ELISA - Technique immunologique - Thaïlande.

## 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<sup>2</sup> 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.

### Serological tests

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

### ELISA

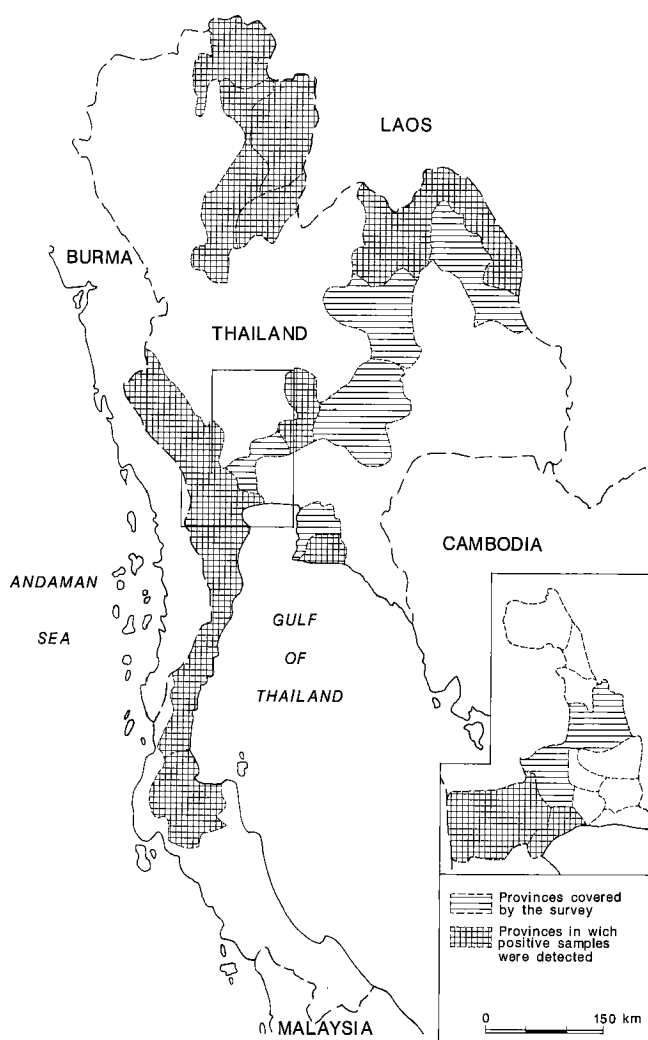
A total of 800 serum samples and 800 corresponding blood eluates were tested using ELISA.

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-

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Reçu 23.11.1990, accepté le 5.3.1991.



Map 1 : Provinces of Thailand covered by the survey.

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 modifications 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  $Kid_{50}/0.025$  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  $NaHCO_3$  (Flow laboratories, Germany). To this were added 5 % or 2 % fetal calf serum, 5 % lactalbumin-hydrolysate solution and 0.2 % gentamycin solution (50  $\mu$ l/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  $\mu$ l of "Enzygnost-Aujeszky" diluent in an incubator 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 procedure. 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 I).

The higher percentage of positive results for serum samples 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.

Sample type	Total	Positive results	Negative results
Serum	800	210 (26.25 %)	590 (73.75 %)
Blood eluate	800	142 (17.75 %)	658 (82.25 %)

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.

Test	Total	Positive results	Negative results
SNT	640	143 (22.3 %)	497 (77.7 %)

## LT

Most of the 182 samples (59 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 » (Ifa-Mérieux) when 59 serum samples and 123 blood eluate samples were tested.

Sample type	Total	Positive results	Negative results
Serum	59	10 (16.9 %)	49 (83.1 %)
Blood eluate	123	53 (43.1 %)	70 (56.9 %)
Total	182	63 (34.6 %)	119 (65.4 %)

**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.

Age of pigs (months)	ELISA serum samples		
	Positive	Negative	Total
0-1	3 (1.43 %)	20	23
1-3	31 (14.76 %)	83	114
3-8	46 (21.90 %)	191	237
> 8	130 (61.90 %)	296	426
Total	210	590	800

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

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, Ifa 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  $\chi^2$  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, 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

We like to extend our special thanks to Dr. T. BHANNASIRI (former Director general of the DLD in Thailand) for his permission to collect the samples and his subsequent support during this process and to Dr. P. ARANYAKANANDA (former Vice Director General of the DLD) and Dr. V. KHUMNIRDPECH for the equally generous advice they gave during this activity. In addition our thanks go to all colleagues at the DLD who assisted us in the collection of these samples.

LEAMCHARASKUL (P.), RENNER-MÜLLER (I.C.E.), REIMANN (M.), MUNZ (E.). A serological survey of the prevalence of Aujeszky's disease antibodies in Thailand using enzyme-linked immunosorbent assays (ELISA), serum neutralization (SN) and latexagglutination (LT) tests. *Revue Elev. Méd. vét. Pays trop.*, 1991, **44** (2) : 135-140

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 - Thaïlande.

LEAMCHARASKUL (P.), RENNER-MÜLLER (I.C.E.), REIMANN (M.), MUNZ (E.). Encuesta serológica sobre la prevalencia de anticuerpos de la enfermedad de Aujeszky en Tailandia mediante el test ELISA y de tests de seroneutralización (SN) y de aglutinación de latex (LT). *Revue Elev. Méd. vét. Pays trop.*, 1991, **44** (2) : 135-140

Se determinó la presencia de anticuerpos de la enfermedad de Aujeszky en los eluatos sanguíneos, sobre papel de filtro, en cerdos tailandeses, mediante los tests ELISA, SN y LT. De un total de 800 muestras examinadas mediante el ELISA, 22 p. 100 de los sueros y 18 p. 100 de los eluatos revelaron resultados positivos. Sobre 640 muestras sometidas a la seroneutralización, debido a una previa respuesta negativa, dudosa o ligeramente positiva al ELISA, 22 p. 100 revelaron resultados positivos. Finalmente, un total de 182 muestras sospechosas fueron igualmente sometidas a aglutinación por latex, 63 de las cuales fueron positivas (35 p. 100). La encuesta revela que entre más viejo es el animal, más grande es la probabilidad de encontrar anticuerpos. Los cuestionarios efectuados a los propietarios tienden igualmente a demostrar que pocos animales fueron vacunados. Estas constataciones, asociadas a la gran frecuencia de anticuerpos detectados, indican que la enfermedad de Aujeszky es un problema común en los cerdos tailandeses. *Palabras claves* : Cerdo - Enfermedad de Aujeszky - Test ELISA - Técnica inmunológica - Tailandia.

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