SERO-PREVALENCE OF AUJESZKY'S DISEASE IN THIRD DIVISION (SIBU) OF SARAWAK

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SUMMARY

Aujeszky's Disease (AD) is a viral disease of economic importance to swine industry in Malaysia. In contrast to Peninsular of Malaysia, in Sarawak, there was no outbreak of AD reported and AD vaccination was not in practice. The previous survey in three other divisions of Sarawak showed 50% of the pig farms were seropositive to AD virus. The present study conducted aimed to determine the sero-prevalence of AD among pig herds and its influencing factors in Third Division of Sarawak. A total of 295 serum samples were collected from 11 farms. The standing pig population of the farms selected represented more than 85% of the total pig population of the division. Sample sizes ranging from 20 to 40 per farm, with the sow/porker ratio of 35%:65% to 50%:50%. A reliable and sensitive indirect ELISA test was developed and employed to test for the presence of specific Aujeszky's disease virus (ADV) in the sera. All the 11 farms were seropositive with the sero-prevalence ranging from 65% to 100% per farm. The percentage of positive samples (90.8%) was confirmed to be significantly higher to the data of the previous survey (34.8%). Most breeders had high antibody titres. In contrast, most porker had low antibody titres. Thus indicated breeders may had been exposed to AD for a longer period compared to the younger porker generation. There was no apparent outbreak as well as no vaccination in practice. Therefore, a high rate of subclinical infection was likely to be endemic in this geographical area. The smaller farm size, longer farm establishment and the use of local source of breeder are variables that contributed significantly to the lower AD prevalence. Remoteness of the pig farm was not found to have an influence on the AD prevalence. Interestingly, minimum biosecurity measures as adopted by some farms were proven to be ineffective as farms without biosecurity had much lower AD prevalence.

Keywords: Pigs, Aujeszky's disease, seroprevalence, Sarawak, ELISA

INTRODUCTION

Aujeszky's disease (AD) had caused multimillion losses each year to the swine industry worldwide. The pig is the only natural host and reservoir for the double-stranded DNA AD virus (ADV). It can also infect cattle, sheep, cats, dogs, and goats with fatal consequences (Fenner *et al.,* 1993). ADV is shed in the saliva and nasal discharges of swine. Like other herpesviruses, ADV can establish latency in the pig and can be reactivated later (Fenner *et al*, 1993). The disease produced, as characterized by nervous symptoms such as staggering gait, 'star gazzing', and somnolence, could cause 100% mortality in preweaning piglets, 40-60% in 4-week-old pigs, 15% in weaned and fattening pigs (Too, 1997). In adults, the disease may produce clinical signs like constipation, abortion, stillbirth, ataxia and convulsions (Dingeldein *et al.*, 1982). In general, sign of central nervous disorder may be seen. Death is usually due to damages in respiratory tract or central nervous system. ADV can cross the placenta at any stage of pregnancy and cause fetal death. Huge economic losses in AD were mainly due to prenatal deaths, abortions, reduced fertility, reduced weight gains and respiratory problems in fattening pigs resulting in severe reduction in productivity (Gustafson, 1984).

A serologic survey showed 77% of the pigs tested in Peninsular of Malaysia were seropositive for AD (Choo *et al.,* 1987). This had been re-confirmed in 1998, 84% of the farms in Peninsular Malaysia was endemic with the disease (Jasbir, 1998a). Another study by Jasbir *et al.* (1998) showed 50% of the farms in 3 divisions of Sarawak namely Kuching, Samarahan and Miri were seropositive with AD. Six of the eleven farms in Kuching Division, 4 of the 7 farms in Samarahan Division and 1 of the 4 farms surveyed in Miri were found to be seropositive to AD virus (Jasbir *et al.,* 1998). A large number of pigs that were found to be seropositive to the field strain of ADV indicating the active cycle of field strain of ADV among the pig population

However no studies was conducted on other 6 divisions of Sarawak state to confirm these findings. Third Division (Sibu) of Sarawak has the third biggest pig population among the 9 divisions in Sarawak. It represented 12.5% of the total pig population in Sarawak. Its production has met the self-sufficiency for the division. Presently the production was destined for local consumption and export to other divisions in Sarawak.

There has not been a survey been conducted in the Third Division of Sarawak. Although there has been no reported clinical outbreak in the division, it was hypothesized that a high rate of AD endemic may occur in many divisions. Therefore, this study was aimed to determine the sero-prevalence of Aujeszky's disease in pig farms in Third Division of Sarawak and to determine the farming practice that may contribute to AD prevalence.

MATERIALS AND METHODS

Farms and sampling

The study focus was in the Third Division of Sarawak where the AD status unknown. Farms were categorized according to the standing pig population (SPP). Farms with SPP less than 300 were categorized as small pig farms, 300 to 1,000 as medium-size pig farms and more than 1,000 were categorized as big pig farms. Out of the 34 farms in Third Division, 11 farms were selected. All 6 of the major farms with standing pig population or SPP >1,000 were included, 2 of the 3 medium (SPP 300-1,000) and 3 small farms (SPP<300) were selected in the study (Table 1). This represents more than 85% of the total pig population in Third Division of Sarawak.

Table 1. Selection of pig farms in the Third Division of Sarawak

Farms	Farm size (SPP*)				
	<100	<300	<1000	<3000	>3000
Available	14	11	3	3	3
Sampled	0	3	2	3	3

*SPP= Standing pig population

Assuming 15% of disease prevalence in a herd with infinity number of animals, a sample size of 19 will give a 95% confidence interval for detecting a positive animal (Smith, 1995). In this study, sample size was exceeding 19 animals. Twenty samples were collected from farms with population of less than 1,000 and 30 samples from farms with SPP 1,000 to 3,000 and 40 samples from farms with pig population of more than 3,000. Percentage of the sample size to the total population was shown in Table 2. Porker to sow ratio samples of 50-65 % and 35-50% (Table 3) were obtained from randomly selected animals in the farm.

Table 2. Farm sizes and number of samples intended

Farm size	Number of samples	Percentage
< 1,000	20	> 2%
1,000-3,000	30	1-3%
> 3,000	40	<1.33%

Serum samples

Blood samples were collected through venupuncture from the jugular vein using Venoject needle. The samples were allowed to clot at ambient temperature overnight. Serum was transferred into 2ml vial and stored at -20∞C.

Questionnaire and data collection

A simple questionnaire was prepared to obtain some useful information from each farm. The questionnaire was completed by interviewing the farmer before or after the sample collection. It was intended to obtain information on location, year of establishment, standing pig population, vaccination status, biosecurity, herd health history, source of breeding animals, hygiene management and feed source. Farms were then categorized based on the questionnaires. Seroprevalence of different category of farms was compared with Pearson Chi-squares analysis.

Enzyme-linked immunosorbent assay (ELISA)

In this study, the ELISA technique by Ali, *et al.* (1999) was used for testing antibodies to AD virus. The ELISA test employed did not differentiate antibodies to field viruses from the vaccine viruses. However, all the farms selected in this study have no history of vaccination to their pigs and presumed have not been exposed to the virus. The ELISA test was carried out with a working volume of 50ml of each reagent. The antigen was diluted in bicarbonate buffer to give a concentration of 10mg per ml antigen protein. Each plate of 96-well (Dynatech. Immulon, USA) was coated with 50ml of the antigen solution, and incubated at 4∞C overnight. The plate was then washed three times with phosphate-buffered saline Tween 20 (PBST). To block non-specific binding, 50ml of 2% BSA-Fraction V (Sigma, UK) was added and the plate incubated at 45∞C for 2 hours. The plate was washed three times as above. Serum was diluted 100-fold. 50ml of diluted sera was added and the plate was incubated at 37∞C for 1 hour. The plate was washed three times. 50ml of pre-diluted goat anti-mouse peroxidase conjugated immunoglobulin (Sigma, UK) was added and allowed to react with antigen bound-mouse antibodies, by incubating the plate again at 37∞C for 1 hour. The plate was washed three times. The 2,2'-Azino-bis (3-ethylbenzthioline-6-sulfonic acid) (ABTS) substrate (Sigma, UK) diluted in citrate-phosphate buffer (CPB) and supplemented with 0.01% of 30% H2O2 was added and the plate incubated for 30-40 minutes at room temperature. Upon completion of the reaction, the plate was read immediately in a spectrophotometer (Dynatech. MR 7000, USA) at dual wave length mode absorbance 410-490nm. Titres of antibodies were determined as described below.

Hyperimmune and negative sera were used as positive and negative controls respectively. All the samples were run in duplication. Mean optical density of the duplicates was taken. Samples were considered to be positive if the optical density (O.D.) was higher than three standard-deviation of the mean O.D. of the negative control. This will give a 99% confidence interval of the results of ELISA test.

Antibody levels were arbitrarily classified into qualitative parameter of negative, weak positive, positive and strong positive. Optical density higher than mean of the negative control plus three standard-deviation of the mean, but lower than 0.200 was considered weak positive. Optical densities equal to or higher than 0.200 but lower than 0.300 was considered to be positive. Optical densities equal to or higher than
0.300 was considered strong positive.

Statistics

Non-parametric statistical analysis of the factors

affecting the different levels of sero-prevalence was done by using Pearson Chi-square. Student's t-test was conducted to determine differences in antibody titres.

RESULTS

Seroprevalence of AD in farms

All 11 farms tested were positive to AD antibodies. High numbers of the samples from each of the farms were seropositive ranging 65% to as high as 100% (Table 3)

Table 3. Number and percentage of positive samples according to farms

Farm	Number of positive sample			Total serum sample	% positive
	Sow	porker	Total		
F1	15	14	29	30	96.7%
F2	10	8	18	20	90.0%
F3	15	19	34	40	85.0%
F4	15	25	40	40	100%
F5	15	14	29	30	96.7%
F6	15	14	29	30	96.7%
F7	5	9	14	20	70.0%
F8	9	10	19	20	95.0%
F9	10	13	23	25	92.0%
F10	8	5	13	20	65.0%
F11	10	10	20	20	100%
Total	127	141	268	295	

Seroprevalence of AD in Sows and Porkers

From the total of 295 serum samples, 268 samples or 90.8% were seropositive. From a total sample of 131 from the sow, there were 127 seropositive samples or a higher proportion of strong positive samples. Porkers have a greater number of negative and weak positive samples, but a lower proportion of strong positive samples. The differences were show in Table 4.

Seroprevalence and biosecurity

There was no farm has a good biosecurity measure. Only 5 farms have a minimum biosecurity set up, as indicated by a proper sanitary practice upon entry to the respected farms, proper fencing, physical outlay of the farm and properly managed animal feed store. There was a significant different ($p<0.05$) of sero-prevalence 96.9%. 141 samples or 86.0% from a total of 164 samples from porker were seropositive (Table 4).

There was a significant difference ($P<0.05$) between the antibody titer of the sow and porker. In sow, there were 40.5% weak positive, 15.3%positive and 41.2% strong positive. In porker, there were 63.4% weak positive, 18.9% positive and 3.7% strong positive. The sow has a lower proportion of negative samples, but between the farms with biosecurity than those without biosecurity measure. Interestingly, however, its correlation against prevalence rate was negative whereby the farms with no biosecurity have a lesser prevalence than the farms with biosecurity (Table 5).

Seroprevalence and farm size

There was a significant difference ($p<0.05$) in seroprevalence and antibody titres between farms of different standing pig population. Smaller farms of less than 1000 SPP have a lower seroprevalence than farms of more than 1000 SPP. The bigger the farm the higher the prevalence. Farms of larger size significantly have a greater and increasing proportion of high-antibody titre samples compared to the smaller size of farms (Table 6).

Seroprevalence and farm location

Farms were categorized into 3 categories. Farms that were near to heavy traffic area and with neighboring farms at close proximity were considered not isolated. Farms which were located quite far from other farms (>1 km) and located at moderate traffic area were consider moderately isolated. Farms which were far from other farms (>5km) and located at place with very low human traffic were considered isolated.

There was no significant ($p<0.05$) effect of the location of the farms on the AD seroprevalence. There was no significant difference observed between farms that were not isolated, moderately isolated or isolated were not different in the prevalence (Table 7)

Seroprevalence and years of farm establishment

Farms were categorized into 3 categories of age as in Table 8. There was a significant different ($p<0.05$) in the effect of the establishment years or farm age to AD ser-prevalence. The farms with >20 years of establishment rank the lowest in prevalence, follow by farms 10-20 years and highest in <10 years. The "younger" the farm, the higher the prevalence.

Seroprevalence and the source of breeders

There was a significant ($p<0.05$) different of the AD prevalence by the source of breeders obtained by farms. Farms who get local breeder supplies have the lowest prevalence, followed by farms that obtained from abroad sources. The highest prevalence was observed in the farms that obtained mixed supplies of breeders from local and abroad (Table 9).

DISCUSSION

This study has demonstrated the important of serologic survey to determine the presence and the prevalence of AD in area that were said to be "free" from the disease. ELISA is a very useful and sensitive test to determine even at low antibody level (Durham *et al.*, 1986). It takes a shorter time to perform, is sensitive and can be used for screening large samples at a time. It has also been approved as a standard test in several countries, and it is used in association with vaccination and AD eradication programs (Fenner *et al.*, 1993).

Table 4. Seroprevalence of AD in porkers and sows

Breeder	Negative Samples	Positive Samples			
		Weak positive	Positive	Strong positive	Total positive
Sow	4 (3.1%)*	53 (40.5%)	20 (15.3%)	54 (41.2)	127 (96.9%)
Porker	23 (14.0%)	104 (63.4%)	31 (18.9%)	6 (3.7%)	141 (86.0%)
Total	27 (9.2%)	157 (53.2%)	51 (17.3%)	60 (20.3%)	268 (90.8%)

* Percentage of samples tested negative or positive

Table 5. Seroprevalence and biosecurity

Biosecurity Measure available	Negative samples	Positive samples			
		Weak positive	Positive	Strong positive	Total positive
No	16 (14.5%)*	58 (52.7%)	21 (19.2%)	15 (13.6%)	94 (85.5%)
Yes	11 (6.0%)	99 (53.5%)	30 (16.2%)	45 (24.3%)	174 (94.0%)

* Percentage of samples tested negative or positive

Table 6. Sero-prevalence of AD and farm size

Farm size (SPP)	Negative Samples	Positive samples			
		Weak positive	Positive	Strong positive	Total positive
<300	8 (13.3%)*	41 (68.3%)	6 (10.0%)	5 (8.3%)	52 (86.7%)
300-1,000	8 (20.0%)	26 (65.0%)	4 (10.0%)	2 (5.0%)	32 (80.0%)
1,000-3,000	4 (4.7%)	50 (58.8%)	15 (17.6%)	16 (18.8%)	81 (95.3%)
>3,000	7 (6.4%)	40 (36.4%)	26 (23.6%)	37 (33.6%)	103 (93.6%)

* Percentage of samples tested negative or positive

Table 7. Sero-prevalence and geographic location

Location	Negative	Positive samples			
		Weak positive	Positive	Strong positive	Total positive
Not isolated	18 (10.0%)*	91 (50.6%)	29 (16.1%)	42 (23.3%)	162(90.0%)
Moderately isolated	7 (7.8%)	48 (53.3%)	19 (21.1%)	16 (17.8%)	83(92.2%)
Isolated	2 (8.0%)	18 (72.0%)	3 (12.0%)	2 (8.0%)	23(92.0%)

* Percentage of samples tested negative or positive

Table 8. Seroprevalence and years of farm establishment

Years of establishment	No of farms	Negative samples	Positive samples			
			Weak positive	Positive	Strong positive	Total positive
<10	2	1 (1.4%)*	34 (48.6%)	19 (27.1%)	16 (22.9%)	69 (98.6%)
10-20	3	8 (9.5%)	54 (63.5%)	8 (9.4%)	15 (17.6%)	77 (90.5%)
>20	6	18 (12.9%)	69 (49.3%)	24 (17.1%)	29 (20.7%)	122 (87.1%)

* Percentage of samples tested negative or positive

Table 9. Seroprevalence and source of breeders

Source of breaders	Negative Samples	Positive Samples			
		Weak positive	Positive	Strong positive	Total positive
Local	15 (15.1%)*	67 (67.7%)	10 (10.1%)	7 (7.1%)	84 (84.9%)
Mixed	2 (3.4%)	32 (53.3%)	12 (20.0%)	14 (23.3%)	58 (96.6%)
Abroad	10 (7.4%)	58 (42.6%)	29 (21.3%)	39 (28.7%)	126 (92.6%)

* Percentage of samples tested negative or positive

Based on the ELISA test conducted, none of the farms tested in this study was serologically free from AD. This finding is differ from previous survey in other parts of Sarawak by which only 50% of the farms in the other 3 main pig producing divisions were positive to AD (Jasbir *et al.,* 1998). The overall seropositive samples were 90.8%, which is significantly higher than previously reported (34.8%) by Jasbir *et al.* (1998) in other divisions. The seroprevalence could be described by several contributing factors.

Porkers have normally been selected as an indicator for active cycle of the virus in the farm. A farm with a high proportion of positive porkers to field ADV is considered as having an active cycle of the virus infection. Sows were selected as they stay for a long time in the farm, they have a longer exposure period which give a higher chance of infection. Porkers selected for the sample collections in the present study were older than 5 months or 20 weeks. This has been well beyond the period where maternal antibodies to AD virus could persist for as long as 15 weeks after birth (Too, 1997). In addition, there was no history of vaccination in practiced. Therefore, our results were not affected by maternal antibodies or antibodies to vaccine virus.

Aerosol transmission between farms is possible within a 2 km radius (Aiello, 1997). However, in this study, farm location and their proximity did not show any effect on the prevalence. This is well understood since pigs were raised by an intensive system in designated areas. Thus suggested factors other than the proximity of farms contributing to herd infection.

Introduction of replacement stock from infected farms may cause an increase in the number of seropositive sows (Jasbir, 1998a). Importation of animals of unknown disease status and lack of quarantine facilities may also have contributed to the problems (Too, 1995). A lower AD prevalence in farms with local breeder than imported breeder was obtained from the study. Breeder source is associated with farm size and how intensive the farm is. Therefore, only bigger rich farms can afford importation of breeder from abroad. Most small farmers practice inbreeding once they get new breeder. This may also reduce the chance of introducing new carrier animals and thus lower AD prevalence as compared to large farms (Jasbir, 1998a).

Management practices to include biosecurity are important in the prevalence of the disease (Wittman and Rziha, 1989). Surprisingly a lower prevalence was observed in farms without biosecurity. This may due to the fact that these farms were family business small size and not very intensive producer. Therefore, biosecurity could not be directly relevant to transmission of AD. However, bigger farms wither higher SPP were in high risk of AD transmission. Therefore, barely minimum biosecurity measures would not be adequate to warrant effective physical control to AD

The larger or more intensive a farm, the higher prevalence it have. This was supported by findings of Thawley and Morrison (1988) where the important determinants of virus persistence were herd size and density at which the sows were maintained. Higher density of pig population increases AD transmission due to the possibility of increase contacts between the pigs.

Years of establishment were associated with farmers experience, improvement or degradation on management, time length of AD endemic status and hygiene of the farm. In general, the longer the time, the higher the chance animals of the farm exposed to ADV. However, a negative correlation was observed in this study where the older the farm the lower prevalence it had. Therefore, farmer's experience in raising pigs may contribute most to the control of infectious diseases, in general. Confounding factor like smaller farm size, lower animal movement with closed-herd breeding may also contributed to the result.

A significant difference was observed between antibody levels of porkers and sows. Many serum samples of sows contained high antibody levels to AD. This was in agreement with the hypothesis where sows have been exposed to AD for a longer period compared to porkers. Such longer exposures gave a higher chance of AD infection and even reactivation and re-infection. Breeding stress may cause recycling of infection thus reinforced immune response to AD virus (Jasbir, 1998). The virus can remain latent and reactivated if there is a stimulus (Wittman *et al.*, 1982). Such repeated exposures in sows would lead to a higher titer of antibody.

Sows and porkers showed a very high prevalence of AD antibodies. Sows and porkers have 96.9% and 80% seropositive samples respectively. The difference was significance and well supported by previous findings (Jasbir 1998a). This phenomenon was also contributed by the time factor where the sow has a longer period of exposure than porker (Jasbir, 1998a). The high prevalence of AD among sows may also act as a direct factor to the

cause of high prevalence among the porkers of the same farm. The infected sows could be the source of further infection as they may shed virus continuously in the farm (Grosse-Beilage, 1994), where the naive porkers or sows may get the infection. Reactivation of latent virus is possible especially in sows and bring to the infection of the other animals in the herd (Smith, 1999).

There was no AD outbreak reported in Sibu throughout the years. But this did not exclude the potential occurrence of subclinical disease that exhibited minimal clinical signs. Subclinical disease may responsible for high seroconversion in the farms, as the data shown a large proportion of positive animals. Subclinical AD infection status in the farm may pose serious economic loss in long run (Oirschot, 1989). Sporadic outbreak in the farm may occur with subclinical infection.

All the farms surveyed did not practice AD vaccination. Some of the farmers claimed they had never heard of the disease. Most farmers had very little knowledge of the AD and AD preventive, control measures. Farmers were not considering control measures as there was no report of an outbreak. The veterinary service did not allow AD vaccination. Currently, vaccine importation must be applied with support from laboratory result certified of disease occurrence. All these factors may contribute to the high prevalence of subclinical AD virus infection among the pig population.

Base on this study it was evidenced, Sibu, the Third Division of Sarawak has a very high prevalence of AD antibodies among farms as well as individual animals of the farm. It is believed that subclinical AD virus infection is wide spread among pig farms in Third Division. Therefore, it is suggested an immediate approval of vaccine importation and vaccination of pigs as a routine practice must be carried out. Although vaccination cannot prevent AD infection but can reduce the economic losses. Mass vaccination would be able to reduce the seroconversion in the herd. Combination of vaccination with good standards of hygiene, testing and culling programme can reduce and eradicate the 'wild' virus infection (Motha *et al.*, 1994). A strict quarantine for replacement stock with serological confirmation is required. A strict biosecurity measure must be implemented. Otherwise, an eradication programme for AD or "AD-free state" in Sarawak state in the future may not be realized if the high seroprevalence of the disease is not reduced (Thawley, 1988).

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RINGKASAN

SERO-PREVALENS PENYAKIT AUJESZKY DI BAHAGIAN SIBU SARAWAK

Penyakit Aujeszky adalah penyakit jangkitan virus yang amat penting dari segi ekonomi kepada industri khinzir Malaysia. Berbeza dengan keadaan di Semenanjung Malaysia, Sarawak tidak berhadapan dengan wabak penyakit ini dan imunisasi untuk penyakit ini tidak dilakukan. Kajian lepas yang telah dilakukan mendapati bahawa 50 % daripada populasi babi di Sarawak adalah sero positif terhadap virus penyakit Aujeszky. Kajian ini dilakukan ke atas populasi babi di dalam Bahagian Ketiga (Sibu) Sarawak untuk menentukan sero-prevalens penyakit Aujeszky dalam ternakan babi, serta untuk mengetahui factor-faktor yang mempengaruhinya. Sejumlah 295 sampel serum telah diperolehi daripada 11 ladang ternakan babi di dalam bahagian ini dan diuji dengan kaedah ELISA. Didapati kesemua 11 ladang babi menunjukkan keputusan sero-positif dengan sero-prevalens menjangkau dari 65 % ke 100 % untuk lading-ladang tersebut. Peratusan sample positif pada tahap 90.8 % adalah tinggi berbanding dengan kajian yang lepas (34.8 %). Kebanyakan haiwan pembaka didapati mempunyai titer antibody yang tinggi berbanding dengan "pedaging". Oleh yang demikian boleh disimpulakn bahawa kadar jangkitan sub-klinikal adalah tinggi untuk populasi babi di kawasan geografi ini.