AUJESZKY'S DISEASE IN PIGS PREVIOUSLY IMMUNIZED WITH AN INACTIVATED VACCINE

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Abstract

An epizootic of Aujeszky's disease (AD) occurred in the Ponggol district in Singapore in 1977/78. An oil-adjuvant, inactivated vaccine was introduced to control the disease. Pigs vaccinated at 2 - 4 weeks of age, with or without a booster at 8 weeks of age, became clinically infected with AD between 3 - 5 months of age in several large commercial farms. The morbidity and mortality were 20 - 40% and 2 - 8% respectively. The lesions of pigs which died appeared to be more severe than hitherto reported or seen for infected pigs of this age without prior vaccination. This led one of the authors to postulate that vaccination with this vaccine had hypersensitized some pigs to the AD virus. In view of the present findings some recommendations on the vaccination and control of AD are discussed.

Aujeszky's disease (AD) or pseudorabies (PR) is an acute and often fatal condition of pigs caused by a virus of the herpes group. Clinical infections of pigs have been reported in England (Mackay *et al.* 1962), Northern Ireland (Gordon and Luke, 1955), Europe (Pritulin *et al.* 1970; Toma *et al.* 1969; Canic, 1969; Borgen *et al.* 1969; Bitsch, 1971; Steek *et al.* 1974; Radnai, 1977; Pensaert *et al.* 1977), New Zealand (Burgess *et al.* 1976) and the United States (Shope 1935; Saunders *et al.* 1963; Olander *et al.* 1966). The earlier reports from the United States indicated that clinical AD primarily affected suckling pigs while the disease in older pigs was usually without clinical signs. More recent reports have described outbreaks in which clinical involvement of older swine has been a prominent feature.

Aujeszky's disease in pigs was first confirmed by virus isolation in Singapore in 1971, the virus being isolated from suckling pigs showing the typical nervous signs of the disease (Lim and Loi, 1975). Since then, an average of 4 cases were diagnosed annually up to 1976. In these cases, the virus appeared to affect only piglets. Although mortality in affected litter was high, the morbidity within affected farms was insignificant to justify control measures other than the culling of affected piglets.

In 1977 and 1978, there was a sudden increase in the incidence of the disease. Twenty-eight (28) outbreaks were reported in 1977, and 139 outbreaks in the first half of 1978 of which more than 140 occurred in the Ponggol district. The clinical manifestation of the disease also changed. Prior to 1977, the ADV affected only piglets. In the 1977/78 epizootic, pigs of all ages were affected. The disease was, however, still most severe in piglets where morbidity and mortality almost reached 100% in some farms. The next major loss was caused by abortions which in some farms affected 90% of pregnant sows. Morbidity in matured boars was also high; many boars developed swelling of the scrotum with subsequent sterility as a sequela. The effects of the ADV in weaners and fatteners were variable with morbidity ranging from 5 - 100% and mortality 0 - 25%. (Ngiam *et al.*, 1978).

An oil-adjuvant inactivated vaccine was introduced in May 1978 in an effort to control the disease. This paper describes the experiences encountered in the field with the use of this vaccine.

Vaccine

The vaccine used was manufactured in France. The vaccine was produced from an ADV isolated in France, grown in the porcine kidney cell line, IBR'S2, inactivated with formaldehyde and

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emulsified in an oil adjuvant (Andries *et al.* 1976; Delagneau *et al.* 1975). Between May and December, 1978, approximately 368,000 doses of the vaccine were imported and distributed to farms.

Vaccination programme

As far as possible, the vaccination programme followed the recommendations of the manufacturer. Sows were vaccinated twice initially at an interval of one to two months with the second vaccination given as close to 30 days before farrow as possible. Booster doses were again given at subsequent pregnancies 30 days before farrow.

Boars were given two injections initially at an interval of 1 - 2 months, and every 6 months subsequently.

In actively infected farms all pigs above 2 weeks were vaccinated with a single dose of 2 ml of the vaccine. Fattening pigs above 5 months of age were not vaccinated for economic reasons, and in infected farms most pigs of this age were already exposed to the field virus. In uninfected farms, pigs were generally vaccinated when they were 4 weeks of age.

In October 1978, a revised programme for piglets in infected farms was recommended. This consisted of a primary dose at 4 weeks followed by a second vaccination at 8 weeks, each pig receiving 2 ml of the vaccine at each vaccination.

Vaccination reaction

A few farms were closely monitored for immediate side reactions due to vaccination. In general, reactions occurred only in a small percentage of pigs. These consisted of slight pyrexia, dullness and anorexia which occurred within 2 days post-vaccination. These pigs recovered within a few days without treatment. Some pigs developed a marked swelling at the site of injection, which subsided after a few days. These reactions were considered to be allergic in origin.

Efficacy of vaccination

The clinical disease apparently subsided since mass vaccination commenced from May 1978. In September of the same year, however reports of clinical AD were received by the Centre. The pigs affected were previously vaccinated at 2 - 4 weeks of age and were by then about 3 - 5 months of age. Other classes and age groups of pigs were unaffected. These included sows, boars, pigs above 5 months/old previously affected and having recovered, and pigs below 3 months of age vaccinated at 2 - 4 weeks of age. The last group of pigs however, did not escape the infection. When they reached the age of 3 - 5 months, they became clinically infected. For the following few months, the clinical course of the disease in an infected farm was predictable. Pigs vaccinated at 2 - 4 weeks of age were clinically ill when they reached the age of 3 - 4 months. In spite of booster injections of the vaccine at 8 weeks of age, the vaccinated pigs also became clinically affected. The morbidity in vaccinated pigs was between 20 - 40% and the mortality was 2 - 8%. The morbidity rate was difficult to ascertain as not all pigs in a pen were ill at the same time.

Clinical signs

Initial clinical signs were dullness, anorexia and pyrexia. Affected pigs tended to huddle in a corner and refused to move when disturbed. The clinical appearance of these pigs seemed to be more severe than that seen in pigs of this age prior to vaccination. Conjunctivitis, manifested by ocular serous discharge and congestion of the sclera and eyelids were often seen, especially in pigs housed in slatted floors. Coughing was occasionally present, but most pigs developed severe dyspnea indicated by deep, abdominal breathing. The skin of affected pigs was often congested, and some pigs developed erythema around their extremities similar to changes seen in swine fever. Nervous signs were rarely seen. Typically, the clinical course in affected pigs ranged from a few days to three or four weeks, when they either died, recovered or were culled. Deaths often occurred within one or two days of onset of clinical signs, or after prolonged illness. In some cases, pigs were found dead without having shown any signs of illness.

Gross pathology

The macroscopic lesions in pigs which died varied depending on the duration of illness. The most common lesions present in all pigs were in the respiratory tract, and cervical and pulmonary lymph nodes.

Pigs which died suddenly invariably were in good condition. The lesions were mainly confined to the respiratory tract and lymphatic system. The lungs were bilaterally enlarged, and had a haemorrhagic appearance indicative of interstitial pneumonia. The enlargement was due to an accumulation of greyish fluid in the interlobular spaces. The oedema imparted a characteristic shining appearance to the lungs. Occasionally, pin-point foci of necrosis less than 1 mm were present on the surface. The necrotic lesions were invariably surrounded by a ring of haemorrhage indicative of an infarct. Pleuritis was present as indicated by the presence of varying amounts of straw-coloured fibrinous fluid in the pleural cavity. The trachea and bronchi were always filled with clear yellowish froth and fluid. Extensive ulcerations filled with yellowish plugs were often present in the upper trachea and larynx. The plugs which were cheesy but never caseous were easily dislodged from the lesions. The tonsils of these pigs were often hyperplastic and congested. Occasional punctate ulcers, filled with yellowish cheesy plugs may also be present in the tonsils. In some pigs the lymphatic ducts in the ventral aspect of the lungs were enlarged and prominent. They had a very hard consistency. The sub-maxillary and pulmonary lymph nodes were enlarged, haemorrhagic, and showed focal necrosis on the surface. The lymph nodes may be two to three times their normal size and were very turgid. Sections of these lymph nodes showed extensive necrosis throughout the parenchyma. Fibrinous pericarditis and peritonitis were often present. The pericardial and peritoneal cavities often contained straw-coloured fibrinous fluid which clotted readily. The blood vessels of the brains were engorged. Other organs were usually congested. Foci of necrosis were commonly seen in the spleen. These varied from 1 mm to 3 mm in diameter. They were also surrounded by a ring of haemorrhage.

Pigs which died after a few days of illness showed the same pathological picture described. In addition, infarcts with necrosis were seen in the kidneys of some pigs. Occasional focal necrosis was also present in the liver of these pigs. Lesions in the upper respiratory tract and nasopharyngeal region were variable. Some pigs had extensive ulcerations in the tonsils but ulcerations were minimal in the larynx of the same pigs. In some pigs no macroscopic lesions were seen in the tonsils or larynx, but necrosis was present either in the nasal mucosa or dorsal pharynx. In chronically-ill pigs extensive adhesions of visceral organs, pleura and pericardium were usually present.

Histopathology

Microscopic examination of lungs from autopsied pigs revealed an interstitial pneumonia. In some lungs bronchopneumonia and haemorrhage with bronchial cellular infiltration were evident. Lymph nodes showed necrotising lymphadenitis, with suppuration in some cases. Sections of brain consistently showed non-suppurative meningo-encephalitis. The heart showed chronic fibrinous pericarditis. Kidneys had acute focal arteritis and infarction in the cortex in some pigs.

Virus isolation

Tonsil swabs taken from clinically-ill pigs, and various organs from autopsied pigs were sent to the Veterinary Laboratory for virus isolation.

Aujeszky's disease virus was isolated from 7 out of 19 swabs taken from the tonsils of sick pigs. The positive swabs were from 3 out of 4 farms.

Aujeszky's disease virus isolation from pig organs was variable. In some pigs, virus could be isolated from all organs including brain, tonsils, lungs, lymph nodes, spleen and kidneys. In others, no ADV could be isolated from any organs submitted. In a few pigs, ADV could only be isolated from single organs such as tonsils, lymph nodes or kidney but not from the other organs.

Bacteriology

Various organs submitted for bacteriological examination were in most cases sterile. Occasionally, *Streptococcus* or *Pasteurella multocida* were cultured from lungs.

Direct smears of lungs with focal necrosis were stained with Giemsa and examined for *Toxoplasma gondii* (Koh *et al.* 1978). No *Toxoplasma* organisms were seen.

Discussion

The control of AD has, for a long time, been through hygiene measures and test and slaughter policies. In many countries where the disease occurs sporadically this is still the recommended approach.

In several parts of Europe where AD is endemic and often occurs in epizootic proportions, research has been centered around various vaccines, both killed and modified live virus vaccines against AD. Several modified live AD virus vaccines have been reported to be effective in controlling AD. These include the Bartha strain (Bartha and Kojnok 1963, McFerran and Dow, 1975) and the 'BUK' strain (Skoda *et al*, 1964). The latter has been modified and used in the U.S.A. recently (Bass, 1978). Although killed vaccines have had the reputation of being ineffective, several reports of new inactivated vaccines have appeared with encouraging results. (Toma *et al.* 1976; Lee and Wilson, 1978; Lukert *et al.* 1978). The results obtained in the field, findings at post-mortem and laboratory examinations described in the present report appear to indicate that the inactivated vaccine used was not very effective in protecting fattening pigs against AD in large commercial farms (more than 5,000 pigs) where the disease is endemic. Losses still occurred in spite of herd vaccinations of breeders and piglets, albeit, these losses were mainly confined to fattening pigs above 3 months of age. It is still too early to evaluate the efficacy of the vaccine in adult pigs and protection of piglets via colostrum, as most of these (adults) were probably already exposed to the field virus before vaccination was introduced.

Reports of the efficacy of this vaccine in Europe have been centered on vaccination of sows which subsequently conferred passive immunity via colostrum to their offsprings (Delagneau *et al.*, 1975; Andries *et al.* 1978; Toma *et al.* 1976). Andries *et al.* (1976) and Toma *et al.* (1976) however, also reported that susceptible fattening pigs vaccinated with this vaccine withstood challenge with a field strain. There are few reports on the effect of vaccination in piglets possessing maternal antibodies. Bass (1978) reported that piglets with maternal antibodies could be successfully immunized with the modified live vaccine, 'BUK' strain, as early 3 weeks of age, whereas 8 of 11 pigs vaccinated at 2 weeks of age survived challenge. McFerran and Dow (1973) reported that piglets from immune sows experimentally infected with ADV at 3 days of age did not produce detectable antibodies up to 80 days postinfection. Toma *et al.* (1976) suggested that vaccination of fattening pigs with the French inactivated vaccine at 2 months of age is enough to protect the pigs during their economic life. Although no serological studies were made at the time of vaccination in May, 1978, it could be assumed that most of the piglets in our commercial farms were derived from sows already exposed to ADV in the 1977/78 epizootic. It is possible that naturally-acquired colostral antibodies had interfered with vaccination thus rendering the vaccine ineffective. We have subsequently carried out serological studies which indicated that no increase in serum neutralizing antibodies could be detected in piglets, with or without detectable antibodies, one month after vaccination with the vaccine (unpublished).

Neutralization of the vaccine could merely render the pigs susceptible to ADV when the remaining colostral antibodies have waned. The consistent pathological lesions of vaccinated pigs wich died appeared to indicate that these pigs were more severely affected than previously reported by other workers (Mackay *et al.* 1962; Olander *et al.* 1966) or seen by the authors in pigs of this age group prior to the introduction of the vaccine. There are three possible reasons for this: (a) the strain of AD virus had become more virulent for pigs of this age, (b) the AD infection was superimposed with another infectious agent and (c) vaccination of pigs had somehow hypersensitized the pigs to the field AD virus.

No further outbreaks of similar severity were seen in the same farms when the practice of vaccination of fatteners ceased. Although these farms have ceased vaccination since January 1979 it may be too early to exclude the possibility that the AD virus had not become more virulent, or that there was a concurrent infection present.

However, it is postulated by one of the authors (JGWK) that the pathological lesions were produced as a result of an aberrant immunological response of some pigs vaccinated with the inactivated vaccine. It is possible that protective colostral antibodies in piglets were able to neutralize the protective antigenic component of the AD vaccine, but not the allergenic component which then stimulated the vaccinate to produce allergic antibodies or cells. Whether this is the case, or whether piglets from non-immune sows could also be sensitized to the same degree requires further investigation. It is of interest to note that Iotov (1973) found that reactions to the intradermal test during the course of AD were strongest at the 90th day post-infection, whereas neutralizing antibody levels reached a peak earlier at 30 - 60 days after infection of pigs. Further, Scherba *et al.* (1978) found that suckling pigs were non-responsive to the skin test even tough they were the progeny of sero-positive sows, whereas 50% of infected pigs with serum neutralizing antibodies reacted to the intradermal test. These reports appear to indicate that at least two immunological responses are stimulated when pigs are exposed to AD virus, one is the production of serum neutralizing antibodies generally considered as indication of immunity, and the second possibly cell-mediated delayed type hypersensitivity detectable by the skin test.

This may be analogous to atypical measles in the medical field. Children previously vaccinated with inactivated measles vaccines who were subsequently exposed to the natural infection developed atypical measles which is more severe than the classical disease (Rauh *et al.* 1965; Fulginiti *et al.* 1967; Nichols, 1979). Fulginiti and Arthur (1969) found that children who had received killed measles vaccine had an almost universal skin test reactivity to measles antigen but almost 2/3 of this group lacked serum antibody. In contrast, children who had measles previously or had been given live measles vaccine had no skin test reactivity and had detectable serum antibodies. They suggested that delayed hypersensitivity was responsible for the altered reactivity in some recipients of killed measles vaccine. It has also been suggested that this type of immunologic imbalance occurs because of an anomalous situation in which the killed vaccine stimulates predominantly serum antibodies but not secretory antibodies. Upon subsequent exposure to the natural virus due to the secretory antibody deficiency, a viral replication can occur within the respiratory tract. Since only limited serum antibodies are available and the viral antigen is in relative excess, antigen-antibody complexes occur, which leads to immunologic injury (Bellanti, 1971).

If the allergy hypothesis is correct future vaccine evaluations should include tests which would detect hypersensitive responses in vaccinates. This would apply more so to modified live virus vaccines which may be perpetuated in the field. It has been reported that dengue shock syndrome occurs as a result of a second infection with heterologous dengue virus and that an immunological mechanism is involved in the pathogenesis of the syndrome (Halstead *et al.* 1957), the primary infection cases being usually so mild that medical care is not sought (Russell, 1970). Recent reports have indicated that the attenuated measles virus vaccine may be capable of sensitizing some individuals (Cherry *et al.* 1972; St. Geme *et al.*, 1976). The aberrant immunological response may not be manifested until the protective antibodies have subsided and rendered the vaccinates susceptible to infection. Until more information is available about this vaccine, and other AD vaccines, it is recommended that the inactivated vaccine should not be given to pigs possessing colostral antibodies. As the colostral antibody level varies from pig to pig, and hypersensitivity probably reaches a peak at about 90 days post-exposure, it is further recommended that vaccination should be done when pigs are 3-1/2 - 4 months of age. Constant monitoring of antibody levels in sows is necessary to estimate the optimum age of vaccination of their progeny. In the meantime, evaluation of other AD vaccines should be accelerated in case this recommendation is not effective.

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Discussion

Shimizu, Y. (Japan): Why did you have such a high incidence of the disease in 1977-1978?

Answer: The high incidence of pseudorabies in 1977-1978 was probably due to the increased numbers of breeding pigs imported from the USA in 1977 and also to the concentration of pigs within a small area, which reached a peak in 1977-1978.

Konno, S. (Japan): I do not think that the lesions you presented are typical of pseudorabies. Was there any contamination?

Answer: I agree with you that the lesions were not typical of classical pseudorabies. We are tempted to call the condition "atypical pseudorabies" analogous to "atypical measles". We were unable to isolate another bacterial, protozoal or viral agent. When vaccination was discontinued in these farms, no cases of death with similar lesions were encountered.

Srihakim, S. (Thailand): (1) In this case of pseudorabies is it possible that hog cholera might be a complication? (2) Did you diagnose pseudorabies by inoculation of tonsils to rabbits? Did you look for the presence of inclusion bodies in the cells of the nervous system (brain for example) or in the kidney cells of the pigs? (3) Did you investigate the possibility of hog cholera?

Answer: (1) and (3). We examined tonsils and brains of these pigs by direct fluorescent antibody staining of cryostat sections along with attempting isolation of hog cholera virus by inoculation of PK-15 cell line. Results for hog cholera were negative. (2) We made the diagnosis of pseudorabies by virus isolation in PK-15 and/or chick embryo fibroblast cell culture as well as by histopathology of target organs. There was evidence that the condition described was associated with pseudorabies infection.