CONGENITAL BOVINE EPIZOOTIC ARTHROGRYPYSIS AND HYDRANENCEPHALY IN AUSTRALIA

Distribution of Antibodies to Akabane Virus in Australian Cattle after the 1974 Epizootic


Introduction

Outbreaks of arthrogryposis and hydranencephaly (AG/HE) in calves in Japan have been associated with the presence of neutralising antibodies to Akabane virus in the sera of affected calves before suckling colostrum (Miura et al. 1974; Kurogi et al. 1975). The 1974 epizootic of AG/HE in south-eastern New South Wales (N. C. Shepherd, C. D. Gee, T. Jessup, G. Timmins, S. N. Carroll, and R. B. Bonner, personal communication) offered an opportunity to determine whether such a correlation occurred in calves before suckling colostrum (Miura et al. 1975; Shepherd, personal communication) and to determine if there was a correlation between the distribution of AG/HE, neutralising antibodies to Akabane virus and the possible arthropod vectors of the virus.

Akabane virus has been isolated in Japan from the mosquitoes Aedes vexans and Culex tritaeniorhynchus (Oya et al. 1961). In Australia, it has been isolated from the biting midge Culicoides brevitarsis twice by Doherty et al. (1972), and also by H. A. Standfast (personal communication) in 1975. Furthermore, the area in which the NSW epizootic occurred (Shepherd et al., personal communication) was adjacent to the southern limit of C. brevitarsis (Murray 1975), suggesting a southerly shift in distribution of the midge took the virus from an endemic situation, where pregnant animals were most likely immune, into an area where the cattle population contained highly susceptible pregnant animals.

A serological survey for the presence of antibodies to Akabane virus was carried out across the epizootic areas of New South Wales, supported by the examination of sera from other states. The aims of the survey were to determine the distribution of Akabane virus in Australia and to determine whether there was a correlation between the distribution of AG/HE and C. brevitarsis. The results of this study are presented in this paper.

Materials and Methods

Virus

The B8935 strain of Akabane virus (Doherty et al. 1972), which successively received 3 passages in mice, 1 in cattle, 2 additional passages in mice, followed by 2 to 4 further passages in cell culture (either Vero or BHK21 cells), was used throughout this study.

Cell Cultures

The virus was grown in either African green monkey kidney (Vero) cells (Westaway 1968) or baby hamster kidney (BHK21) cells (French and St. George 1965). The Vero cells were grown in Medium 199 (M199) containing 5% foetal calf serum and maintained in M199 containing 1% foetal calf serum for tube cultures or M199 containing 0.2% bovine serum albumin and buffered with 15mM HEPES at pH 7.5 for Microtitre cultures.

Neutralisation Tests

Serums were heated at 56°C for 30 min before assaying for neutralising antibodies to Akabane virus using Vero cells. Equal volumes of serum or diluted serum were mixed with virus and incubated for 1 h at room temperature before inoculation into either tube or Microtitre cultures. The tests were read after 4 days incubation at 37°C. The antibody titres are expressed as the reciprocal of the initial dilution of serum that, after mixing with virus, neutralised the virus in 50% of the tubes or wells (Reed and Muench 1938) inoculated at that dilution. A serum was taken as positive for antibody if it prevented a viral cytopathic effect (CPE) in all tubes when used undiluted, or in all Microtitre wells when used at a dilution of 1 in 2. Controls carried out with each test included the titration of virus and known negative and positive serums.

Tube tests were performed using 100 50% tissue culture infective doses (TCID50) of Akabane virus per tube, 4 tubes being inoculated with 0.2 ml of incubated virus-serum mixture. The tubes were rolled during incubation.

In the Microtitre tests two-fold serial dilutions (3 to 4 wells/dilution) were prepared in 0.025 ml of maintenance medium per well in transfer plates || using 0.025 ml dilutors. Twelve to 25 TCID50 units of virus (in 0.025 ml) were added to each well and the plates incubated. Maintenance medium (0.2 ml/well) was added to confluent monolayers of Vero cells (seeded

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the previous day at 4 x 10^4 cells/well) in flat-bottom tissue culture Microtitre plates each containing 96 wells. The incubated serum-virus mixtures were transferred to this tissue culture tray by capillary action through the holes in the bottom of the transfer plate. Tissue culture and transfer plates were re-used as follows: the plates were decontaminated overnight in a 0.1% sodium hypochlorite solution, washed and soaked in distilled water, and sterilised by immersion in 70% alcohol followed by UV irradiation.

The Survey

The epizootic (April to October, 1974) occurred in south-eastern New South Wales, principally on the south coast around Bega and extending north-west towards Yass and Goulburn (Shepherd et al., personal communication; Figure 2). Blood samples, usually from groups of 50 animals, were obtained during September, 1974, till February, 1975, from the following 4 main transects across the epizootic area (Figure 2): Wollongong on the coast to Wentworth in the far west of New South Wales (A); Nowra to Muttama (B); Braidwood to Wagga Wagga (C); and Bega over the Snowy Mountains to Tumbarumba and along the Murray River to Echuca (D). Other samples were obtained from properties to the south of Narrandera, New South Wales, and in Victoria, Western Australia and Tasmania. These were supplemented by samples collected from sentinel herds (St. George et al 1973) in Tasmania, New South Wales, Queensland, Northern Territory, South Australia, Western Australia, and Papua New Guinea.

Results

Neutralisation Tests

Seventy-five to 100% CPE was visible in both tube and Microtitre Vero cell cultures 4 days after infection. The cells in the tube cultures were usually infected 4-5 days after they were seeded into the tubes. Within the range of 3 to 5 days, the age at which these cells were used did not appear to have a significant effect on the results. In contrast, the Microtitre assay results were very dependent upon the age of the cells. Monolayers prepared 24 h previously gave clear and reproducible results, which compared well with the tube assay system. Cell monolayers older than 2 days were found to give unsatisfactory results as they were 10- to 100-fold less sensitive to Akabane virus and more non-specific degeneration was also observed. The Microtitre assay was easier and faster to perform than the tube assay. The test was very economical requiring less reagents and the plates could be re-used at least 10 times. The antiserum controls showed that both tests gave very consistent results, with less than a 2-fold variation of the titre of the positive sera in over 100 tests. The neutralising antibody titres of sera collected from cattle in the epizootic area ranged from 2 to 512, the majority being between 4 and 32.

Survey of Cattle Serums for Neutralising Antibodies to Akabane Virus

The results of the survey for the presence of neutralising antibodies to Akabane virus in cattle sera in Australia are illustrated in Figure 1. The detail in the map (Figure 2) is of the epizootic area in which transects from east to west were made to determine the limits of Akabane virus spread in south-eastern Australia. Details of the results illustrated in Figures 1 and 2 are presented below.

Transects Across Epizootic Area in New South Wales

Wollongong to Wentworth — There was a high prevalence of antibodies in animals at Wollongong (48/50, 96%). West of this area the prevalence of positive reactors gradually fell in herds at Robertson (25/50, 50%), Taralga (14/47, 30%), and Wagga (1/10, 10%), then increased again around Leeton (19/69, 28%) in the Murrumbidgee Irrigation Area, and then rapidly declined, being 1/10 (10%) at Darlington Point and negative further to the west at Hay, Narrung and Wentworth.

Nowra to Muttama — The prevalence was high at Nowra (36/44, 82%). The prevalence declined slightly to a fairly constant level at Goulburn (40/73, 55%), at Collector (24/50, 48%), at Yass (45/100, 45%) and at Muttama (29/50, 50%) on the Murrumbidgee River.

Braidwood to Wagga Wagga — The prevalence at Braidwood (21/50, 42%), Queanbeyan (19/50, 38%) and Tumut (18/50, 36%) was constant, but was very low at Wagga Wagga (1/50, 2%).

Bega to Echuca — The prevalence of animals possessing antibodies in the Bega area was very high (203/226, 90%). There was also a high incidence of AG/HE in calves born in this area (Shepherd et al, personal communication). An intensive survey was therefore made of this area. The prevalence and distribution of animals possessing antibody was as follows: Brogo (41/41, 100%), Bholic (40/47, 85%), Buckajo (41/48, 85%), Timbillica (11/14, 79%), Wandello (47/51, 92%) and Tilba (23/25, 92%). With an increase in elevation in the Great Dividing Range, the prevalence fell markedly at Cathcart (2/21, 10%), Cooma (1/51, 2%) and Tumbarumba (1/47, 2%). Further west and along the Murray River, no positive animals were detected.

Elsewhere in New South Wales

A small number of serum samples from suspected arthrogypotic calves born south of Nar-
randera had no antibodies to Akabane virus. There were a number of positive serum samples from Trangie (5/12, 42%). Serum from a deformed calf born at Gunnedah in 1974 had antibody to Akabane virus. A number of sheep flocks were bled and the results of testing these sera for antibody are described later.

Victoria

All sera from animals inland of the Dividing Ranges were found to be negative. There was a number of animals possessing antibody in the east Gippsland area. At Genoa there were 6/9 (67%) animals with antibody. Animals at Mallacoota (0/4), Leongatha (0/33) and Melbourne (0/23) were negative.

South Australia

Sera from two herds on the Murray River were negative for antibodies to Akabane virus. A number of animals possessing antibody in the Ranges were found to be negative. There was a high percentage (0/33) in all areas surveyed except for 1/7 at Northam (the history of this positive animal was uncertain). Of interest are the negative animals found at Yuna Station (0/15), near Geraldton, as this was the most northerly herd sampled in this survey and is situated just south of the known distribution of C. brevitarsis (Figure 1).

Tasmania

Sporadic cases of arthrogrypotic calves occur in Tasmania (B. Munday, personal communication). Sera from some affected calves and their dams were tested and were found to have no detectable antibody (0/20). Sera from cattle in a sentinel herd at Cressy were also found to be negative.

South Western Australia

There were reports (V. Smith, personal communication) of minor outbreaks of congenitally deformed calves in herds at Albany and at Esperence. Sera were obtained from these herds and a small survey of about 400 animals was made in the south-western area of Western Australia. The sera were found to be negative in all areas surveyed except for 1/7 at Northam (the history of this positive animal was uncertain). Of interest are the negative animals found at Yuna Station (0/15), near Geraldton, as this was the most northerly herd sampled in this survey and is situated just south of the known distribution of C. brevitarsis (Figure 1).

Northern Australia

A high percentage (100/125, 80%) of sera from cattle in the CSIRO sentinel herds (maintained in cooperation with State Departments of Primary Industry) in the north of Western Australia, in the Northern Territory, and in Queensland were found to possess neutralising antibodies to Akabane virus. The distribution of these herds is illustrated in Figure 1. Of particular interest are the animals with antibody found in the Alice Springs area (12/20, 60%) in September 1975.

Although these sera were collected after the main survey period, they did cause us to test sera previously collected from this area in 1966 and 1973. A small number (2/29, 7%) of these were also found to be positive.

Papua New Guinea

Sera from sentinel cattle (maintained in cooperation with the Department of Agriculture, Stock and Fisheries) at Boroka (0/11), Lae (0/16) and Goroka (0/20) did not possess neutralising antibodies to Akabane virus.

Studies on Serums from Other Species

Sheep — There was a high prevalence of antibody in some sheep flocks in the endemic and epizootic areas. Sheep from Charleville, in Queensland (9/27, 33%) and Badgery's Creek (52/85, 61%) and Bringelly (46/50, 92%) in New South Wales were positive. Sheep from Trundle (0/50), Temora (0/83), and Crookwell in New South Wales (0/12), from Seymour (0/20) in Victoria, and Cressy (0/30) in Tasmania, were negative. A flock of antibody-negative sheep has been maintained at Bringelly since November, 1974, and monthly bleedings of 50 sheep up to June, 1975, have shown no seroconversion. This suggests that Akabane virus was not active in this area over the 1974/75 summer season.

Other Species — A small number of pig sera from Goulburn were tested, and 1/8 was positive. A small number of horse (0/3) and human (0/10) sera from Bega were also negative. The significance of these limited results is questionable. At Beatrice Hill, Northern Territory, where an intensive arbovirus study is currently in progress, neutralising antibodies to Akabane virus have been found in sera from buffaloes (16/16) but not from wallabies (0/28), domestic fowls (0/14, bled weekly), or pigs (0/6). At the Flinders River in Queensland, a very high proportion of sera from horses (19/20) were found to be positive.

Discussion

A good, but not absolute, correlation between calves suffering from epizootic arthrogryposis and hydranencephaly and the presence in their serum of neutralising antibodies to Akabane virus has been demonstrated in Australia (Hartley et al 1975), similar to that reported by workers in Japan (Miura et al 1974; Kurogi et al 1975). The survey described in this paper was carried out to determine the distribution within Australia of antibodies to Akabane virus in 1974 and
the relationship of this to possible vector distribution in the period.

The survey was designed to transect (Figure 2) the area in and around where the 1974 epizootic of AG/HE occurred in south-eastern New South Wales. This included the known (Murray 1975) southerly distribution of C. brevitarsis, a biting midge from which the virus has been isolated in Australia on 3 occasions (Doherty et al 1972; H. A. Standfast, personal communication). The high prevalence of positive sera from these areas at Sydney and into northern Australia (Figure 1) could be consistent with the virus being endemic in these areas. The periodic epizootics of AG/HE in south-eastern New South Wales (Hartley et al 1974) suggests that the virus spreads out of areas where it is normally enzootic into areas where fully susceptible pregnant cattle are available for infection. The survey demonstrated (Figure 2) infections of cattle by Akabane virus, at least during the summer of 1974, extended outside the known distribution area for C. brevitarsis. This could have resulted from an extension of C. brevitarsis outside its known distribution due to unusual environmental conditions, or involvement of another vector, which if normally present in these areas, does not appear to transmit Akabane virus regularly. Although there was no available evidence in support of, or against, the southerly extension of C. brevitarsis in the summer of 1974, it is known that C. brevitarsis was abundant around Sydney because of the numerous complaints about "Queensland itch", an intense dermatitis resulting from bites of C. brevitarsis. Furthermore, as C. brevitarsis breeds in cattle dung, and as the warm humid weather of the summer extended beyond March into May, conditions would have favoured its proliferation. However, an intensive survey in this area of the insect distribution, seasonal abundance, and feeding habits, together with serological monitoring of cattle and sheep, needs to be undertaken to determine the vector(s) of Akabane virus in epizootics.

There was a good correlation of Akabane antibody in cattle with the distribution of C. brevitarsis, in the north and west of Australia. Antibodies were present in the sentinel herd at Derby, in northern Western Australia, but absent in sera from cattle and some calves with deformities in the south-west of Western Australia. This correlation suggests that C. brevitarsis may indeed be a principal vector of Akabane virus in Australia. However, the presence of cattle with antibody at Alice Springs suggests that C. brevitarsis is possibly not the only vector for the virus. This midge has not been found at Alice Springs (Murray 1975)* and other possible vectors should be investigated for this area and elsewhere in Australia, particularly as the virus has been isolated from mosquitoes in Japan (Oya et al 1961).

One area of particular interest is around and south of Narrandera, in the Riverina, where C. brevitarsis has not been found (Murray 1975). Clearly, Akabane virus was active in the irrigation areas around Leeton, but its activity appeared to decrease rapidly to the south-west. It was absent along the Murray River. Other arbovirus vectors were very active during January to April, 1974, throughout the Murray River area, there being epidemics of Murray Valley encephalitis and Ross River virus infections (J. Forbes, personal communication). These viruses were isolated from the mosquito, Culex annulirostris (Marshall and Woodroffe 1974; Doherty et al 1974). In Japan, Akabane virus was isolated (Oya et al 1961) from the mosquitoes Aedes vexans and Culex tritaeniorhynchus. If mosquitoes had played an active role in the transmission of Akabane virus in south-eastern Australia in 1974, it is reasonable to expect that antibodies would have been found in sera from cattle in the Murray Valley area. Likewise, if Culicoindes marksi, which is known to bite stock and occurs throughout Australia (Murray 1975), had played a role, then antibodies should have been detected in sera collected from a wider area than observed in this survey (Figure 1).

Another area of interest is Papua New Guinea. The absence of cattle with antibody in Papua New Guinea suggests that Akabane virus might not be found there. However, the survey is too small to be of significance for this area and further study would be necessary to establish that Akabane virus is not present.

It is suggested that arthrogryposis and hydranencephaly in cattle occurs as an epizootic disease along the margin of the distribution of C. brevitarsis in New South Wales and is caused by Akabane virus moving from an endemic situation, into populations of susceptible cattle, as the distribution of the vector changes. A small percentage, about 20%, of animals tested in the endemic area, Northern Australia, were found not to possess neutralising antibodies. A number of these susceptible animals could become infected with Akabane virus and produce the

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*The original survey was done from 1968 to 1970. In a more recent survey by H. A. Standfast (personal communication) in February 1976, following a number of good seasons, C. brevitarsis was found at 2 locations near Alice Springs. These findings suggest the possible extension southwards of C. brevitarsis under these conditions.
sporadic and isolated cases which are observed in this area. It must also be emphasised that not all congenital defects characterised by arthrogryposis or hydranencephaly are caused by Akabane virus. This is especially true of cases seen in Tasmania and in the south of Western Australia, where it was not possible to incriminate Akabane virus as the cause. Other infectious agents, possibly viruses, and other factors may be involved and warrant further investigation.

A further consideration of importance is the presence of antibodies to Akabane virus in sheep. In excess of half the sheep in flocks at Bringelly and Badgery's Creek, to the west of Sydney, being positive. It has now been demonstrated (Parsonson et al. 1975) that Akabane virus can cause congenital defects in lambs characterised by AG/HE, micrencephaly, and porencephaly. Indistinguishable lesions from those produced experimentally, have been seen in 20 lambs born in 1971 and 1973 at Bringelly and in 1973 a high prevalence (4/5) of neutralising antibodies to Akabane virus was found in these lambs (Hartley and Haughey 1974; W. J. Hartley, K. G. Haughey and A. J. Della-Porta, unpublished data). A congenitally deformed kid (AG/HE) has also been produced (Inaba et al. 1975) by infection of a pregnant goat with Akabane virus. The virus thus infects cattle, sheep, and goats, a situation akin to bluetongue. C. brevitarsis will bite both cattle and sheep (M. J. Muller and M. D. Murray, unpublished data) and is considered to be conspecific with C. pallidipennis (W. Wirth, cited by Standfast and Dyce 1972), a known vector of bluetongue in Africa. An understanding of the epidemiology of Akabane virus may give us an appreciation of the likely movements of bluetongue should it enter Australia.

Summary

At the end of the 1974 epizootic of bovine congenital arthrogryposis and hydranencephaly in south-eastern New South Wales, an Australia-wide serological survey (about 4,000 serums) was made to determine the distribution of cattle possessing serum neutralising antibodies against Akabane virus. Eighty per cent of the serums from cattle at Boroka, Lae, and Goroka born in Tasmania and south-western Western Australia were not associated with the presence of Akabane virus. In Papua New Guinea, serums collected from cattle at Boroka, Lae, and Goroka did not possess neutralising antibodies. The distribution of cattle possessing antibodies in Australia would fit a spread of the virus by Culicoides brevitarsis, a biting midge from which Akabane virus has been isolated on three occasions. The possibility of other vectors, as well as C. brevitarsis, was suggested by the presence of cows possessing antibodies at Alice Springs, where this biting midge has not been found. Possibly most cattle in northern Australia become infected early in life. The epizootics in New South Wales could occur when seasonal conditions allow a southerly extension of virus-infected C. brevitarsis which feed on susceptible pregnant animals. C. brevitarsis also bites sheep, and both neutralising antibodies to Akabane virus and congenitally deformed lambs have been observed in the epizootic area. An understanding of the distribution of Akabane virus and C. brevitarsis, a possible Australian vector for bluetongue virus, may prove useful if bluetongue should enter Australia.

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References


BOOK REVIEW

A FIRST PROCEEDINGS BOOK

Three papers on anaesthesiology were Use of Muscle Relaxants in Anaesthesia in Small Animal Practice (E. R. Parsonson), Anaesthesia for the Injured Animal (M. S. Pass), Reflex Hazards During General Anaesthesia (M. A. E. Rex).


Problems concerned with cattle included papers on Distribution of Antibiotics to Akabane Virus in Australian Cattle after the 1974 Epidemic (A. J. Della-Porta, M. D. Murray and D. H. Cybinski), Pathology of Akabane Disease in Cattle (W. J. Hartley and W. G. de Saram), Akabane Virus: Experimental Infection in Cattle and Sheep (I. M. Parsonson, A. J. Della-Porta and W. A. Snowdon), Problems Associated with Artificial Breeding in Beef Cattle: a Review (I. Knight), Egg Transfer in Cattle (J. N. Shelton), New South Wales Mastitis Control Programme (R. T. Hoare and R. F. Sheldrake), Pathogenesis of Bovine Mastitis with Special Reference to the Role of Selective Tissue Adhesion (A. J. Frost and D. D. Wanasinghe), Glutathione Peroxidase in the Diagnosis of Selenium Deficiency (D. J. Paynter and J. W. McDonald), Infectious Bovine Rhinotracheitis Virus as a Cause of Bovine Infertility (W. A. Snowden, I. M. Parsonson and D. P. Dennett), Some Nutritional Factors which Affect the Fertility of Dairy Cattle (A. P. Hunter), Farmer-Veterinarian Attitudes to One Another (H. M. Jones), the Break Even

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