SOME ARTHROPOD–BORNE VIRUSES INFECTING LIVESTOCK IN AUSTRALIA

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Introduction

Australia is fortunate that it is free of the major arthropod-borne diseases affecting livestock in many other countries of the world. However, in recent years a large number of arboviruses have been isolated in Australia (Doherty *et al.* 1972, Doherty *et al.* 1973) but only a small number appear to be capable of causing disease. Bovine ephemeral fever virus was isolated in 1968 (Doherty *et al.* 1969), Akabane virus 1968 (Doherty *et al.* 1972) and Aino virus 1968 (Doherty *et al.* 1972). Bluetongue virus (BT 20) was isolated in 1975 (St. George *et al.* 1978) but no disease has been attributed to this virus in any animal species under field conditions.

Bluetongue (BT) Virus

History of BT20 virus

A systematic investigation that was initiated at Beatrice Hill in the Northern Territory of Australia in October 1974 by the CSIRO Division of Animal Health had the following objectives:

(i) provide information on the seasonal distribution of arthoropods attacking livestock in the far north of the Northern Territory.
(ii) provide insects for the isolation of arboviruses; in particular to try and identify the arthropod vector(s) of bovine ephemeral fever virus and
(iii) collect sera from sentinel cattle, buffaloes and native fauna and test them for antibody to a range of viruses.

A virus, designated CSIRO 19, was isolated from a pool of 214 *Culicoides* representing an estimated 11 species, amongst which were *C. actoni, C. brevitarsis, C. bundyensis, C. marksi, C. perigrinus,* and *C. schulzei* (St. George et al. 1978). The virus was isolated in tissue cultures of BHK 21 cells, and was subsequently identified as belonging to the BT group at the Yale Arbovirus Research Unit in the USA. Subsequently, the virus was tested at the Veterinary Research Institute, Onderstepoort, South Africa for its relationship to known members of the BT virus group. It was confirmed that the virus belonged to the BT virus group, but that it was not one of the known serotypes, although an antiserum prepared against BT-20 cross reacted to some extent with BT serotypes 4 and 17 (Erasmus. B., personal communication). The virus was subsequently classified as BT virus serotype 20.

Distribution of BT 20 virus in Australia

Following the identification of BT 20 virus a survey was undertaken using a microtitre serum neutralization (SN) test (St. George *et al.* 1978) to determine the distribution of BT 20 virus in northern Australia. By testing sera collected from cattle in sentinel herds (St. George *et al.* 1977) and held in serum banks by CSIRO, and sera collected specifically for the purpose, it was demonstrated that the virus was, or had been, active in restricted areas in northern Australia, i.e. the Kimberley region of Western Australia, the northern area of the Northern Territory, and the Cape York Peninsula in Queensland. Evidence of infection was found in cattle and buffaloes, all outside the sheep raising areas, and no evidence of infection was detected in sheep. Two important questions that were asked by the disease control authorities were how long had the virus been present in Australia and was the virus pathogenic for sheep? The earliest indication of BT 20 virus

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infection in Australia was the latter half of 1973 with sero-conversion in sentinel herds occurring between October 1973 and July 1974 (St. George T.D., personal communication).

Pathogenicity of BT 20 virus for sheep and cattle

There is no evidence that BT 20 virus produces any signs of clinical disease in sheep, cattle or buffaloes under field conditions (Snowdon and Gee 1978). The virus has been tested for pathogenicity in sheep and cattle under experimental conditions when varying degrees of pathogenicity were demonstrated. The 9th or 10th passage of the virus grown in suckling mice and BHK 21 cells was inoculated into three sheep and passaged a further twice in sheep at the Veterinary Research Institute at Onderstepoort, South Africa. There was a mild febrile response in the first passage sheep with complete absence of other clinical signs. The 2nd passage sheep had a brief, but definite, febrile response and mild mouth lesions, and the 3rd passage sheep had a more pronounced febrile reaction but still only mild mouth lesions (Erasmus. B., personal communication). It was concluded that BT 20 virus, at the passage level used in these experiments, was of low pathogenicity for sheep compared to other serotypes under similar conditions.

St. George and McCaughan (1979) infected sheep with BT 20 virus from three sources;

(i) After inoculating sheep with 3rd passage virus grown in BHK 21 tissue cultures, 10 sequential sheep passages were carried out by the intravenous inoculation of blood collected at the febrile peaks. A total of 45 sheep was inoculated.
(ii) Ten sheep were inoculated with virus grown for six days in mosquitoes of the species *Culex annulirostris* or *Aedes aegypti*.
(iii) Twenty sheep were inoculated with homogenates prepared from pools of infected *Culicoides* of various species.

Most sheep had a febrile response ranging from 39.9°C to 42.1°C. Four sheep showed no clinical signs other than fever, the remaining sheep being mildly ill for one day, or moderately or severely ill for two to seven days. The most frequent clinical signs were mild to moderate conjunctivitis and stomatitis, hyperaemia of the bare skin of the inner thighs, and mild coronitis, followed by banding of the hooves. There were no deaths.

Six cattle were inoculated intravenously with BT 20 virus, three being inoculated with 5th passage virus grown in BHK 21 tissue cultures, and the remainder being inoculated with blood collected from the first passage animals during viraemia. The cattle showed no febrile response or other clinical signs.

Australia wide serological surveys for bluetongue virus activity

With the relatively free passage of cattle from northern Australia to virtually all States, it was necessary to determine whether BT 20 had been introduced into other areas and if it had been, had it spread to other susceptible animals in those areas. Large numbers of cattle and small numbers of buffaloes had been introduced into southern areas of Australia from the Northern Territory and north Queensland between the latter half of 1973 and the beginning of 1978.

When surveys were undertaken only rarely did cattle and buffaloes from northern Australia have SN antibody to BT 20, and furthermore, where positive reactors were found there was no serological evidence of spread to locally bred cattle and sheep. This could have meant that the animals were not viraemic when introduced into these areas, or that if they were viraemic, suitable vectors were not available to transmit the virus.

To speed up the surveys for antibody to BT in all Australian states, the agar gel precipitin (AGP) test, developed at the Veterinary Research Station Glenfield, New South Wales (Littlejohns I.R., personal communication) was widely used, and the complement fixation (CF) test developed at the CSIRO Animal Health Research Laboratory, Parkville, Victoria (McPhee D, French, E.L. and Snowdon, W.A., unpublished data) was used to a limited extent. A significant number of sera collected from cattle in NSW, Queensland, Northern Territory and northern Western Australia gave positive reactions in the AGP test with a small proportion reacting in the CF test. Only a very small number of AGP positive reactors were found in sheep. Of those animals with positive AGP

reactions that were born and bred outside northern Australia, none contained SN antibody to BT 20 virus. Although large numbers of cattle and smaller numbers of sheep, were surveyed in Victoria, Tasmania, South Australia, and southern Western Australia only sporadic AGP positives, of no epizootiological significance, were found.

The significance of positive AGP and CF reactors

Ninety two sera, collected from all States of Australia, with the exception of Victoria and Tasmania, and selected on the basis of their positive reaction in the AGP test, and negative SN antibody status to BT 20, were tested at the Animal Virus Research Institute, Pirbright, UK against BT serotypes 1 to 17, BT 20, Ibaraki virus and the virus of epizootic haemorrhagic disease (EHD) of deer. A number of these sera (50%) had low titres of SN antibody to a number of BT serotypes and/or Ibaraki virus but the remainder (41%) were negative for antibody to all viruses tested (Della Porta, A.J., Sellers, R., Herniman, K., Littlejohns, I.R., Smith, V., St. George, T.D., Snowdon, W.A., and McPhee, D., unpublished data).

In general the SN antibody titres obtained would not permit a positive diagnosis of previous BT virus infection to be made. Instead the only conclusion that could be made was that cattle, and occasionally sheep, had been infected with agents that could have belonged to the BT virus group, or which were antigenically related to it.

It was obvious that the only way the agents producing the AGP reactions to BT virus could be identified was to isolate and identify them. During the 1979 summer and autumn sentinel herds were established in NSW, Queensland and the Northern Territory with the specific aim of isolating these agents. Although a number of agents have been isolated (St. George, T.D., Littlejohns, I.R., Gard, G., personal communication) and some appear to be orbiviruses, their relationship to the BT virus group has yet to be determined.

Arthropod vectors of BT 20 virus

A knowledge of the arthropod vectors of BT 20 virus is important in understanding the natural history of BT 20 virus in Australia, and making projections of the possible limits of its spread in cattle and/or sheep populations. Preliminary results of studies aimed at determining which *Culicoides* species would support the replication of BT 20 virus were reported by Standfast *et al.* 1978. *Culicoides* were collected by truck and light trap (Dyce *et al.* 1972) and fed on sheep that had received BT 20 virus and were viraemic at the time. Those *Culicoides,* that had taken a blood meal, were held for 6-8 days and subsequently processed and inoculated into BHK 21 tissue cultures and sheep to detect the presence of virus. Of five species of *Culicoides*, *C. actoni*, *C. brevitarsis* and *C. schultzei* supported replication of BT 20 virus, whereas *C. marksi* and *C. bundyensis* did not. Further studies are in progress to determine whether biological transmission of BT 20 virus could be obtained with those *Culicoides* species that supported its growth.

It is significant that *C. brevitarsis* supported the growth of BT 20 virus as it has long been considered a potential vector of BT virus (Murray 1975). *C. brevitarsis* which is also probably the major vector of Akabane virus (Della Porta *et al.* 1976) has a distribution across northern Australia extending into the south eastern areas of the continent (Murray 1975). *C. schultzei* occurs in the Northern Territory (Murray 1975) and *C. actoni* is known to occur in the Northern Territory, and the Cape York Peninsula and coastal areas of Queensland as far south as Brisbane (Standfast *et al.* 1978).

Discussion

Serological surveys have shown that BT 20 virus has a limited distribution in northern Australia and it provides a unique opportunity to study its activity on an annual basis and determine whether it is likely to spread to areas in southern Australia. The significance of the presence of animals, outside the areas where BT 20 virus occurs, with AGP antibodies to BT 20 virus, but not SN antibodies, is not known. Studies are currently underway to isolate the agents producing these

reactions so that their true relationship to the BT virus group and other orbiviruses can be determined.

The importance of BT 20 virus infection in Australia is related to its effects on the export of livestock and livestock products, since the virus does not produce clinical disease under field conditions and, in its present distribution, appears to be of no economic importance whatsoever. Some countries that have traded with Australia for many years placed temporary embargoes on the importation of livestock products including wool. Such embargoes have no scientific basis, their being no evidence that under field conditions BT virus infection can be transmitted in any other way than by arthropod vectors.

Bovine Ephemeral Fever Virus

Introduction

Major epizootics of bovine ephemeral fever (BEF) occurred in Australia in 1936 - 37, (Seddon 1938), 1955 - 56 (Seddon 1966), 1967 - 68 (Gee *et al*. 1969) and 1970 - 71 (St. George *et al.* 1977). These outbreaks were characterized by the spread of the disease over hundreds of miles in a relatively short period of time; the rapid spread being most likely due to virus infected insects being carried by the prevailing winds (Seddon 1938; Murray 1970). BEF is enzootic in certain areas of Australia between major epizootics (Snowdon 1971, St. George *et al.* 1977) although large areas of southern Australia are free of disease between major epizootics; and there are other areas where the disease has not occurred.

BEF virus

BEF virus isolates from Japan, South Africa, Australia, Nigeria, Kenya, and Iran, appear to be antigenically closely related (Inaba *et al.* 1969, Lecatsas *et al.* 1969, Kemp *et al.* 1973, Davies and Walker 1974, Hazrati *et al.* 1975). Two BEF viruses isolated from mosquitoes caught in northern Australia differed to some extent when compared in cross neutralization tests in tissue cultures with the Australian reference strain QEF/BB7721 (Standfast *et al.* 1976 a), but cattle that received these viruses were subsequently shown to be resistant to challenge with the EF/1956 strain of virus which is indistinguishable in cross neutralization tests to the type strain, QEF/BB7721 (Snowdon 1970).

BEF virus has properties that place it in the rhabdovirus family. In culture it produces infective bullet shaped virions as well as cone shaped defective-interfering particles that are not infective (Lecatsas *et al.* 1969, Theodoridis and Lecatsas 1973, Ito *et al.* 1969, Murphy *et al.* 1972, Della Porta A.J., Smale, C.J. and Brown, F., unpublished data). Some confusion has existed as to the form of RNA in BEF virus. Tanaka *et al.* 1972 reported that the virus contained a 12S piece of double stranded RNA which was different to other members of the rhabdovirus group. Della Porta and Brown (1979), however, demonstrated that BEF virus possessed single stranded 42 RNA, and the defective interfering particles single standed RNA of approximately 18-20S, similar to the prototype virus of the rhabdovirus family, vesicular stomatitis virus-Indiana.

Arthropod vectors of BEF

The method of spread of BEF in Australia, right from the first major epizootic in 1936 - 37, suggested that insects were involved in the transmission of the disease (Seddon 1938). However it has not as yet been possible to determine the vector(s) involved in the massive spread of the virus across Australia in a relatively short space of time. Although a major attempt was made to isolate BEF virus from both mosquitoes and midges during the 1967 - 68 epizootic (Doherty *et al.* 1972, Doherty *et al.* 1973) the virus was not isolated. More recently two isolations of BEF virus have been made from mosquitoes caught in northern Australia (Standfast *et al.* 1976). One isolate was obtained from a mixed pool that included 4 *Culex (Lophoceraomyia)* species, 4 *Uranotaenia nivipes*, 1 *Uranotaenia albescens* and 1 *Aedes (Verralina) carmenti*, and the other from a pool of 77 *Anopheles (Anopheles) bancroftii*. After consideration of the distribution and seasonal abundance of these

species it was concluded that they were unlikely to be important vectors in the epizootic spread of BEF. BEF virus has been isolated from a mixed pool of *Culicoides* species in Kenya (Davies and Walker 1974), and Standfast *et al.* (1973) have recovered virus from *C. brevitarsis* and *C. marksi* eight days after feeding virus, but not at 2, 4, or 6 days, thereby increasing speculation as to their role in the transmission of the disease.

Immunity to BEF

Animals known to have recovered from infection with BEF virus are able to resist infection for long periods (Snowdon 1971), and live vaccines prepared from virus that has had only a small number of passages in cell culture, or suckling mice, are also effective in producing immunity without producing clinical disease (Heuschele and Johnson 1969; Theodoridis *et al.* 1973, Tzipori and Spradbrow 1973, Inaba *et al.* 1974, Spradbrow 1975, Tzipori and Spradbrow 1978). However, repeated passage of BEF virus in laboratory systems results in loss of pathogenicity and immunogenicity for cattle (Inaba *et al.* 1969, Heuschele and Johnson 1969, Snowdon 1970). Inaba *et al.* 1973 produced a formalin inactivated, aluminium phosphate gel-adsorbed vaccine that produced levels of neutralizing antibody and resistance to challenge in cattle given two doses of vaccine. Work carried out in Australia (Della Porta and Snowdon 1979) has demonstrated that high titres of neutralizing antibody could be produced using a vaccine prepared from b-propiolactone inactivated virus in Freund's incomplete adjuvant but that there was little correlation between the levels of antibody and protection against challenge with virulent BEF virus. The passage level of the virus did not affect the antibody response as virus passaged 27 times produced very high antibody levels. In addition, virus that had received 7 passages, and was then made into inactivated ($10^{7.2}$ PFU) or live ($10^{4.7}$ PFU) vaccines produced similar neutralizing antibody responses after two vaccinations, although 0/4 of the cattle that received the inactivated vaccine resisted challenge whereas, 3/4 of those that received the live vaccine were resistant. These results suggest that levels of neutralizing antibody to BEF virus are not directly related to resistance, and that T-cell dependence and cell mediated immunity may be involved as has been suggested for rabies (Turner 1976, Kaplan *et al.* 1975, Wiktor *et al.* 1977).

Akabane Virus

Introduction

Epizootics of arthrogryposis and hydranencephaly (AG/HE) have occurred in cattle in Australia for many years (Blood 1956, Bonner *et al.* 1961, Hartley and Wanner 1974, Shepherd *et al.* 1978). Japanese workers (Miura *et al.* 1974, and Omori *et al.* 1974) provided the first evidence of the association of Akabane virus with the disease when they found neutralizing antibodies to Akabane virus in pre-suckling serum samples collected from affected calves. Subsequently Hartley *et al.* 1975 confirmed that a similar association occurred between the disease and the virus in Australia.

Importance of Akabane disease

Akabane disease is probably of very little economic importance in areas where the virus is enzootic, as cattle, the most commonly affected species, experience infections with the virus early in life and by the time they are of breeding age they are immune. However, when infections occur outside the enzootic areas, significant losses can occur. During the 1974 epizootic in south eastern New South Wales losses of calves affected with AG/HE were in excess 4-5000 and if abortions and still-births were included, the losses, based on the Japanese experience, could have been as high as 15,000 animals (Anon 1975).

Host range

In Australia Akabane disease has been recognised in cattle (Blood 1956, Whittem 1957, Hartley and Wanner 1974) and sheep (Hartley and Haughey 1974, Della Porta *et al.* 1977) and

neutralizing antibody to Akabane virus has been found in cattle, sheep, goats, horses, buffaloes and camels (Doherty *et al.* 1972, Della Porta *et al.* 1976 and Cybinski *et al.* 1978). Akabane virus has been isolated from *Culicoides brevitarsis* (Doherty *et al.* 1972, St. George *et al.* 1978), from Akabane disease affected ovine foetuses (Della Porta *et al.* 1976), and from the blood of a clinically normal bull (St. George *et al.* 1977).

Arthropod vectors of Akabane virus

The only arthropod from which Akabane virus has been isolated in Australia is the biting midge *C. brevitarsis* (Doherty *et al* 1972, St. George *et al.* 1978). The distribution of Akabane virus, based on the presence of neutralizing antibody in cattle, is similar to the distribution of *C. brevitarsis* (Cybinski *et al.* 1978, Della Porta *et al* 1976). Outbreaks of Akabane disease occur in south eastern Australia when the environmental conditions during summer and autumn permit the spread of *C. brevitarsis*, and hence Akabane virus, out of the enzootic areas and into areas containing susceptible pregnant cattle and sheep (Della Porta *et al.* 1976).

Akabane virus has been isolated from the mosquitoes *Aedes vexans* and *Culex tritaeniorhynchus* in Japan (Oya *et al.*) and from *Anopheles funestus* in Kenya (Metselaar and Robin 1976). The virus has also been shown to grow in *Aedes vigilax* but not *Culex annulirostris* after feeding on infected blood (Kay *et al.* 1975).

References

1) ANON. (1975): Akabane Virus. CSIRO, Div. Anim. Hlth. Annu. Rep. 1975.
2) BLOOD, D.C. (1956): Arthrogryposis and hydranencephaly in newborn calves. *Aust. Vet. J.* **32**, 125.
3) BONNER, R.B., MYLREA, P.J. and DOYLE, B.J. (1961): Arthrogryposis and hydranencephaly in calves. *Aust. vet. J.* **37**, 160.
4) CYBINSKI, D.H., ST. GEORGE, T.D. and PAULL, N.I. (1978): Antibodies to Akabane virus in Australia. *Aust. vet. J.* **54**, 1.
5) DAVIES, F.G. and WALKER, A.R. (1974): The isolation of ephemeral fever virus from cattle and *Culicoides* midges in Kenya. *Vet. Rec.* **95**, 63.
6) DELLA PORTA, A.J., O'HALLORAN, M.L., PARSONSON, I.M., SNOWDON, W.A., MURRAY, M.D., HARTLEY, W.J. and HAUGHEY, K.J. (1977): Akabane disease: Isolation of the virus from naturally infected ovine foetuses. *Aust. vet. J.* **53**, 51.
7) _____, MURRAY, M.D. and CYBINSKI, D.H. (1976): Congenital bovine epizootic arthrogryposis and hydranencephaly in Australia. Distribution of antibodies to Akabane virus in Australian cattle after the 1974 epizootic. *Aust. vet. J.* **52**, 496.
8) _____ and SNOWDON, W.A. (1979): An experimental virus vaccine against bovine ephemeral fever. 2. Do neutralizing antibodies protect against infection? *Vet. Microbiol.* In press.
9) _____ and BROWN, F. (1979): The physico-chemical characterization of bovine ephemeral fever virus as a member of the family *Rhabdoviridae*. *J. Gen. Virol.* In press.
10) DOHERTY, R.L., CARLEY, J.G., STANDFAST, H.A., DYCE, A.L., KAY, B.H. and SNOWDON, W.A. (1973): Isolation of arboviruses from mosquitoes, biting midges, sandflies and vertebrates collected in Queensland, 1969 and 1970. *Trans. R. Soc. trop. Med. Hyg.* **67**, 536.
11) _____, _____, _____, _____, _____, (1972): Virus strains isolated from arthropods during an epizootic of bovine ephemeral fever in Queensland. *Aust. vet. J.* **48**, 81.
12) _____, STANDFAST, H.A. and CLARK, I.A. (1969): Adaptation to mice of the causative virus of ephemeral fever of cattle from an epizootic in Queensland, 1968. *Aust. J. Sci.* **31**, 365.
13) DYCE, A.L., STANDFAST, H.A. and KAY, B.H. (1972): Collection and preparation of biting midges (Fam *Ceratopoganidae*) and other small Diptera for virus isolation. *J. Aust. ent. Soc.* **11**, 91.

14) GEE, R.W., HALL, W.T.K., LITTLEJOHNS, I. and SNOWDON, W.A. (1969): The 1967-68 outbreak of ephemeral fever in cattle. *Aust. vet. J.* **45**, 132.
15) HARTLEY, W.J. and HAUGHEY, K.G. (1974): An outbreak of micrencephaly in lambs in New South Wales. *Aust. vet. J.* **50**, 55.
16) _____ and WANNER, R.A. (1974): Bovine congenital arthrogryposis in New South Wales. *Aust. vet. J.* **50**, 185.
17) _____, _____, DELLA PORTA, A.J. and SNOWDON, W.A. (1975): Serological evidence for the association of Akabane virus with epizootic bovine congenital arthrogryposis and hydranencephaly syndromes in NSW. *Aust. vet. J.* **51**, 103.
18) HAZRATI, A., HESSAMI, M., ROUSTAI, M. and DAYHIM, F. (1975): Isolation of bovine ephemeral fever virus in Iran. *Arch. Inst. Razi.* **27**, 81.
19) HEUSCHELE, W.P. and JOHNSON, D.C. (1969): Bovine ephemeral fever. II. Responses of cattle to attenuated and avirulent virus. *Proc. Annu. Meet. U.S. Anim. Hlth. Assoc.* **73**, 185.
20) INABA, Y., KUROGI, H., SATO, K., GOTO, Y., OMORI, T. and MATUMOTO, M. (1973): Formalin-inactivated, aluminium phosphate gel adsorbed vaccine of bovine ephemeral fever virus. *Arch. Gesamte. Virusforsch.* **42**, 42.
21) _____, _____, TAKAHASHI, E., SATO, K., OMORI, T., GOTO, Y., HANAKI, T., YAMAMOTO, M., KISHI, S., KODAMA, K. and HARADA, K. (1974): Vaccination of cattle against bovine ephemeral fever with live attenuated virus followed by killed virus. *Arch. Gesamte. Virusforsch* **44**, 121.
22) _____, TANAKA, Y., OMORI, T. and MATUMOTO,M. (1969): Serological relation between bovine epizootic fever and ephemeral fever. *Jpn. J. Microbiol.* **13**, 129.
23) _____, _____, SATO, K., ITO, H., OMORI, T. and MATUMOTO, M. (1969): Bovine ephemeral fever. III. Loss of virus pathogenicity and immunogenicity for the calf during serial passage in various host systems. *Jpn. J. Microbiol.* **13**, 181.
24) ITO, Y., TANAKA, Y., INABA, Y. and OMORI, T. (1969): Electron microscopic observations of bovine epizootic fever virus. *Natl. Inst. Anim. Hlth. Q. (Tokyo)* **9**, 35.
25) KAPLAN, M.M., WIKTON, T.J. and KOPROWSKI, H. (1975): Pathogenesis of rabies in immunodeficient mice. *J. Immunol.* **114**, 171.
26) KAY, B.H., CARLEY, J.G. and FILLIPPICH, C. (1975): The multiplication of Queensland and New Guinean arboviruses in *Culex annulirostris* (Skuse) and *Aedes vigilax* (Skuse) (Diptera: *Culicidae*) *J. Med. Ent.* **12**, 279.
27) KEMP, G.E., MANN, E.D., TOMORI, O., FABIYI, A. and O'CONNOR, E. (1973): Isolation of bovine ephemeral fever virus in Nigeria. *Vet. Rec.* **93**, 107.
28) LECATSAS, G., THEODORIDIS, A. and ELS, H.J. (1969): Morphological variation in ephemeral fever virus strains. *Onderstepoort J. vet. Res.* **36**, 325.
29) METSELAAR, D. and ROBIN, Y. (1976): Akabane virus isolated in Kenya. *Vet. Rec.* **99**, 86.
30) MURPHY, F.A., TAYLOR, W.P., MIMS, C.A. and WHITFIELD, S.G. (1972): Bovine ephemeral fever virus in cell culture and mice. *Arch. Gesamte. Virusforsch.* **38**, 234.
31) MIURA, Y., HAYASHI, S., ISHIHARA, T., INABA, Y., OMORI, T. and MATUMOTO, M. (1974): Neutralizing antibodies against Akabane virus in pre-colostral sera from calves with congenital arthrogryposis-hydranencephaly syndrome. *Arch. Gesamte. Virusforsch.* **46**, 377.
32) MURRAY, M.D. (1970): The spread of ephemeral fever of cattle during the 1967-68 epizootic in Australia. *Aust. vet. J.* **46**, 77.
33) _____ (1975): Potential vectors of bluetongue in Australia. *Aust. vet. J.* **51**, 216.
34) OMORI, T., INABA, Y., KUROGI, H., MIURA, Y., NOBUTO, K., OHASHI, Y. and MATUMOTO, M. (1974): Viral abortion arthrogryposis hydranencephaly syndrome in cattle in Japan 1972-74. *Bull. Off. int. Epiz.* **81**, 447.
35) OYA, A.T., OKUNO, T., OGATA, T., KOBAYASHI, I. and MATSUYAMA, T. (1961): Akabane a new arbovirus isolated in Japan. *Jpn. J. Med. Sci. Biol.* **14**, 101.
36) ST. GEORGE, T.D., STANDFAST, H.A., CHRISTIE, D.G., KNOTT, S.G. and MORGAN, I.R.

(1977): The epizootiology of bovine ephemeral fever in Australia and Papua New Guinea. *Aust. vet. J.* **53**, 17.

37) _____ and McCAUGHAN, C.I. (1979): The transmission of the CSIRO 19 strain of bluetongue virus type 20 to sheep and cattle. *Aust. vet. J.* **55**, 198.

38) _____, STANDFAST, H.A., CYBINSKI, D.H., DYCE, A.L., MULLER, M.J., DOHERTY, R.L., CARLEY, J.G., FILIPPICH CHERYL and FRAZIER, Christina, L. (1978): The isolation of bluetongue virus from *Culicoides* collected in the Northern Territory of Australia. *Aust. vet. J.* **54**, 153.

39) _____, CYBINSKI, DAISY and PAULL, N.I. (1977): The isolation of Akabane virus from a normal bull. *Aust. vet. J.* **53**, 249.

40) _____, STANDFAST, H.A. and CYBINSKI, D.H. (1978): Isolation of Akabane virus from sentinel cattle and *Culicoides brevitarsis*. *Aust. vet. J.* **54**, 558.

41) SEDDON, H.R. (1966): Diseases of Domestic Animals in Australia. Part 4. Protozoan and Virus Diseases, 2nd Ed., Rev. by H.E. Albiston, Dept. Hlth., Canberra, *Aust. Dept. Hlth., Vet. Hyg., Serv. Publ. No.* **8**.

42) _____ (1938): The spread of ephemeral fever (three day sickness) in Australia in 1936-37. *Aust. vet. J.* **14**, 90.

43) SHEPHERD, N.C., GEE, C.D., JESSOP, T., TIMMINS, G., CARROL, S.N. and BONNER, R.B. (1978): Congenital bovine epizootic arthrogryposis and hydranencephaly. *Aust. vet. J.* **54**, 171.

44) SNOWDON, W.A. (1970): Bovine ephemeral fever: the reaction of cattle to different strains of ephemeral fever virus and the antigenic comparison of two strains of virus. *Aust. vet. J.* **46**, 258.

45) _____ (1971): Some aspects of epizootiology of bovine ephemeral fever in Australia. *Aust. vet. J.* **47**, 312.

46) _____ and GEE, R.W. (1978): Bluetongue virus infection in Australia. *Aust. vet. J.* **54**, 505.

47) SPRADBROW, P.B. (1975): Attenuated vaccines against bovine ephemeral fever. *Aust. vet. J.* **51**, 464.

48) STANDFAST, H.A. and DYCE, A.L. (1972): Arthropods biting cattle during an epizootic of ephemeral fever in 1968. *Aust. vet. J.* **48**, 77.

49) _____, MURRAY, M.D., DYCE, A.L. and ST. GEORGE, T.D. (1973): Report on ephemeral fever in Australia. *Bull. Off. Int. Epizoot.* **79**, 615.

50) _____, ST. GEORGE, T.D., CYBINSKI, D.H., DYCE, A.L. and McCAUGHAN, C.A. (1978): Experimental infection of *Culicoides* with a bluetongue virus isolated in Australia. *Aust. vet. J.* **54**, 457.

51) _____, _____, DOHERTY, R.L., DELLA PORTA, A.J. and SNOWDON, W.A. (1976): *CSIRO, Div. Anim. Hlth. Annu. Rep.*

52) _____, _____ and DYCE, A.L. (1976): The isolation of ephemeral fever virus from mosquitoes in Australia. *Aust. vet. J.* **52**, 242.

53) TANAKA, Y., INABA, Y., SATO, K., OMORI, T. and MATUMOTO, M. (1972): Double strandness of ribonucleic acid of bovine ephemeral fever virus. *Jpn. J. Microbiol.* **16**, 95.

54) THEODORIDIS, A., BOSHOFF, S.E.T. and BOTHA, M.J. (1973): Studies on the development of a vaccine against bovine ephemeral fever. *Onderstepoort J. vet. Res.* **40**, 77.

55) _____ and LECATSAS, G. (1973): Variation in morphology of ephemeral fever virus. *Onderstepoort J. vet. Res.* **40**, 139.

56) TURNER, G.S. (1976): Thymus dependence of rabies vaccine. *J. Gen. Virol.* **33**, 535.

57) TZIPORI, S. and SPRADBROW, P.B. (1978): A cell culture vaccine against bovine ephemeral fever. *Aust. vet. J.* **54**, 323.

58) _____ and _____ (1973): Studies on vaccines against bovine ephemeral fever. *Aust. vet. J.* **49**, 183.

59) WHITTEM, J.H. (1957): Congenital abnormalities in calves; arthrogryposis and hydranen-

cephaly. *J. Path. Bact.* **45**, 574.
60) WIKTOR, T.J., DOHERTY, P.C. and KOPROWSKI, H. (1977): *In vitro* evidence of cell mediated immunity after exposure of mice to both live and inactivated rabies virus. *Proc. Natl. Acad. Sci. U.S.A.* **74**, 334.

Discussion

Inaba Y. (Japan): Among the three materials of bluetongue virus (BT 20 virus) you used, which one is the most pathogenic for sheep?

Answer: I believe that the virus grown in various species of *Culicoides* was the most pathogenic.

Inaba Y. (Japan): What do you think of the overwintering survival of BT 20 virus in Australia?

Answer: An understanding of the overwintering process for BT 20 virus will have to await the results of further studies on the vectors of the virus. One of the potential vectors, *C. brevitarsis* is known to breed all the year round and could indicate one mechanism of the overwintering process.

Inaba Y. (Japan): When was the BT 20 virus introduced to Australia and where from?

Answer: We do not know when BT 20 virus was introduced into Australia or from where. No evidence has been produced to show that BT 20 virus occurs in any country outside Australia. The earliest indication of BT 20 virus infection was obtained from studies in sentinel cattle in Northern Australia between October 1973 and July 1974.

Oya A. (Japan): Are there any fluctuations in the emergence of *C. brevitarsis* in Australia depending on the years?

Answer: There are probably yearly fluctuations in the emergence of *C. brevitarsis* in Australia depending on the environmental conditions. However studies over a number of years would be required to demonstrate such fluctuations. For example, the heavy rains which occurred in the Central part of Australia in 1974 may have resulted in shifting the distribution of *C. brevitarsis* to more southern areas as more cattle were put to graze, hence producing larger amounts of dung for *C. brevitarsis* to breed on.

Inaba, Y. (Japan): Has anyone succeeded in establishing colonies of *C. brevitarsis*?

Answer: Establishment of colonies has not been achieved. Colonies raised in the laboratory may not be representative of populations in the field owing to selection. It would be a good tool for carrying out basic studies on arthropod-virus interactions. However, extrapolation from results obtained in the laboratory to field conditions may lead to erroneous conclusions. Also, in transmission studies mortality rate of *C. brevitarsis* may be high after the first feeding on sheep.