Epidemic bovine ephemeral fever and arbovirus surveillance on a dairy farm in south-east Queensland 1973-1987

NT Hunt, DH Cybinski, SS Davis, EJ Harris, MJ Muller, TD St.George and H Zakrzewski

(presented at the 11th Australian Arbovirus Symposium 9-14 September 2012, Surfers Paradise Queensland)

The genesis of the longitudinal study was the offer by Terry Hunt to participate in the CSIRO sentinel herd scheme devised by Toby St.George. Many scientific papers and an uncertain number of higher degree theses derive from the 22 arboviruses that were isolated from the cattle or insects collected there.

This paper reviews the field study of successive epidemics of natural bovine ephemeral fever. Important differences from the experimental disease were noted but not solved for a number of years. The periods of daily observations and collection of samples, while resident, were carried out by Neville Hunt or Steven Davis.

Many of the arboviruses that were isolated from cattle or insects were not relevant to the core studies and were not fully investigated beyond the demonstration that they were new to Australia.

The paper was written by Toby St.George, who held the supporting publications, but it also derived from direct discussions between us. The recent upsurge in the study of novel arboviruses has called on our personal recollections and unpublished data to satisfy the requests for background information of novel viruses discovered there.

I presented the paper at the symposium because of my familiarity with the farm and the derivative virology and entomology.

NT Hunt

We concur: Steven Davis

Toby St.George
INTRODUCTION

Bovine ephemeral fever (BEF) is caused by a rhabdovirus (BEFV) spread by flying vectors. Epidemics occurred in Australia in 1936-1937, 1955-1956, 1967-1968 and 1971. Another epidemic had begun in Queensland and was moving south by April 1973 (St.George et al 1973). Attempts thus far in Australia to isolate BEFV from any insect had been unsuccessful. In 1939, an excellent study of BEF under experimental conditions was carried out on dairy cows by Mackerras et al (1940).

Most information published on the clinical course of the disease was based on experimental disease. Our principal objectives were to study the natural course of the disease in cows during an epidemic of BEF and to isolate BEFV from insect vectors in the vicinity.

PEACHESTER DAIRY FARM

In 1973, it became possible to have access to the dairy cows on a commercial farm located approximately 100 km north of Brisbane, Queensland. The farm was 60 hectares of cleared rain forest on a tributary of the Brisbane River, with tree-lined edges, flowing through it. The climate is sub-tropical, with most of the 1800 mm of rain falling in the summer between September and April. The 80-100 dairy cows were milked at 5 am and 4 pm. Most calvings occurred in the February to April period. The herd was in located at 152.53 E 20.51 S, at 150 metres altitude in an area where it was hoped that the 1973 BEF epidemic would pass through and provide a presence of BEFV in the. Local insect vectors.

BOVINE EPHEMERAL FEVER

In the BEF epidemics of 1973, 1976, 1981-1982 and 1984, periods of daily samplings were carried out selected groups of cattle on the Peachester farm. An observation protocol was established at Peachester to follow the course of epidemic BEF through a group of 12 cows. Blood samples for haematology and biochemistry plus virus isolation in suckling mice, were taken from the jugular vein once daily. Insect collections by means of light traps and truck traps were used for species identification and virus isolation.
1973 BEF EPIDEMIC

The first case of BEF occurred on 20 November and the last on 13 December. A group of 12 heifers aged 12-18 months were bled daily for 3-14 days before becoming ill with BEF and for 7 days after illness. All experienced BEF and made complete recoveries. Mild signs of illness, a tendency to depression and loss of appetite were observed approximately half a day before severe clinical signs. These animals were found to have an elevated rectal temperature in the mild stage. This confirmed farmers' observations that they could pick the next animals to experience BEF by the change of behaviour.

The haematology showed a clear correlation between a neutrophilia, high fibrinogen level (18g/litre), and a fall of plasma calcium to a mean of 1.95 mM/litre on the day of severe illness. The unexpected finding was that there was pre-existing neutralising antibody to BEFV before clinical illness. This was present in 11 of the 12 heifers on the first day of sampling, in all the available serum samples collected 3-10 days before clinical signs. On the day of severe illness the titre fell to almost undetectable levels in all and rose sharply to above initial levels on the day following the severe illness (St.George et al 1984). A considerable puzzle was created by the presence of specific BEFV non-protective antibodies up to 10 days ahead of the onset of disease in the cows. This is illustrated in Fig 1. This lead to the plan to collect serums a long time before the next epidemic occurred.

BEF Antibodies

![Graph showing the titre of neutralising antibody to BEFV present before clinical disease fell in the days before clinical disease on day 0.](image)

Figure 1 the titre of neutralising antibody to BEFV, present before clinical disease fell in the days before clinical disease on day 0. It rose anamnestically in the following days.
Poor clot contraction was evident during the clinical illness. (St.George 2000) This caused considerable problems in obtaining sufficient serum for neutralization tests that required 1 ml of serum at that time, before the minaturisation era.

1976 BEF EPIDEMIC

A sentinel group of group of 12 heifers, replaced annually, was established in 1974, 1975 and 1976. They were bled monthly. None of these heifers had experienced BEF, nor were there any neutralising antibodies in their serum in 1974 and 1975. No clinical BEF occurred in these years. In 1976, another BEF epidemic was anticipated. The sentinel group was bled monthly May to October, then weekly from 19 November to 30 December, and daily from 3 January to 28 February 1976. The pattern of antibodies to BEFV being present prior to BEFV infection was repeated in 8 of the 12 sentinels. In individual animals, BEFV antibodies were detected 15 to 63 days before clinical signs occurred. Unlike primary infections following clinical BEF, the titres were 8 or lower. The epidemic began on 29 January and finished on 15 February.

Again, the titre of neutralising antibodies fell on the day of severe illness and rose sharply the following day. The haematology and biochemistry was similar to that found in the 1973 epidemic. A strain of BEFV (CSIRO 1820) was isolated from a cow’s blood on 29 January 1976. There was no knowledge of the existence of Kimberley virus at this time. A rhabdovirus CSIRO 368, later found to be a strain of Kimberley virus (KIMV), was isolated at Long Pocket laboratories in February 1980, from the blood of a sentinel cow in the Northern Territory (Cybinski and Zakrzewski 1984). At a later stage, the KIMV neutralization test was applied to the 1973 and 1976 daily blood samples. There was sufficient lead time in representative series to show that antibodies to KIMV appeared 17-90 days before they experienced clinical BEF. Antibodies to both KIMV and BEFV fell on the day of acute illness and rose sharply the following day as shown in Fig 2. This knowledge was in place by the time of the 1981-1982 BEF epidemics at the Peacheester farm. The same pattern of neutrophilia and fall of plasma calcium occurred on the day of illness as in the 1973 epidemic. The interval between the appearances of antibodies to KIMV and subsequently to KIMV prior to the BEF epidemic was 3-4 weeks.
Figure 2 Antibodies to KIMV and BEFV in serial serum samples collected from a cow on the Peachester farm prior to and after the 1976 BEF epidemic (St.George et al 1984).

1977 BEF EPIDEMIC

An epidemic of BEF began in October of 1977. However, on the 28 October, the presence of BTV was recognized in Australia and almost all research plans were cancelled. The exception was the filming of clinical cases for the US Department of Agriculture Exotic Diseases series. The footage of the finished film included cases on the Peachester farm. The sentinel group was bled monthly but the primary aim changed to detecting BTV group antibodies.

1981-1982 BEF EPIDEMIC

A program of insect collections was in place from September 1981 to April 1982. *C.brevitarsis* formed 99% of the biting midges. But no single species of mosquito dominated the mosquito collections. There was no clear evidence of seroconversion of any sentinel cattle from the end of the 1977 epidemic until 1981. The traces of BEFV antibodies that developed in sentinel cow sera were later matched to seroconversion to KIMV. Another daily series of blood and potential vector collections was planned for when the approaching BEF epidemic reached the Peachester farm. By this time, the isolation methods for BEFV had improved considerably by the use of *Aedes albopictus* insect cell tissue cultures as they had for a wide spectrum of other arboviruses. There were two complications. The first was that the epidemic arrived in the Christmas shutdown period. The second was that torrential rain on the morning of 13 January washed away the breeding places of potential vectors and this was followed by a river flood in the afternoon that cut the farm in two and restricted road access for 2 days. The BEF epidemic began on 29 December 1981 in the sentinel group (and the herd) on 29 January. There were 10 cattle with severe BEF and two with mild signs. The epidemic was suddenly halted by the flood and torrential rain.
From the collections of blood made before the flood, 10 isolations of BEFV, one each of KIMV, Akabane virus and 112 of an orbivirus (CSIRO Village virus) were made. There was clear evidence of mutual interference between BEFV and CSIRO Village viruses. Seroconversion occurred in this period to Aino, Douglas, Tinaroo, Tibbongargan and Bunyip Creek arboviruses. Only the cattle, from which BEFV was isolated, experienced BEF. The remainder was susceptible in the next epidemic in 1984. (St George 1985). Seven isolations of BEFV were made from cattle blood. This was in contrast to experimental disease where many more isolations were expected during fever. (Uren et al 1992). The blood samples taken during epidemic and natural clinical BEF had pre-existing heterotypically generated antibodies that bind BEFV. Thus the chance of isolating virus was reduced.

The biochemistry and haematology in these cattle was described separately and confirmed a neutrophilia and raised plasma calcium in those cattle that had BEF in contrast to those that did not (Uren and Murphy 1985). These remain the only studies of the biochemistry of natural BEF.

1984 BEF EPIDEMIC

A program of bi-weekly insect collection into alcohol for taxonomy plus updraft traps and animal baited traps for day and night biters were put in place in 1983 although an epidemic of BEF was not expected that year. The next epidemic occurred in January-February 1984. Daily bleedings of cattle and large collections of insects were made as in earlier epidemics. Aspiration of insects biting cattle was added as a technique. The live insects were held to digest blood meals before being tested for arboviruses. BEFV was isolated from Anopheles bancroftii and C. brevitarsis collected on 1 February 1984. Isolations were made of BEFV from two sentinel cow bloods taken on 21 and 28 February 1984 (Cybinski and Muller 1990). Isolates of bunyaviridae, reoviridae, rhabdoviridae and togaviridae were made using BHK 21 and AA tissue cultures and Ae. aegypti live mosquito inoculations. A total of 11 different arbovirus isolations was made from insects collected during the course of this BEF episode (St.George 1985).

Two further isolations of BEFV were made from clinical cases in 1988. The intensive field studies involving cattle came to an end as insect proof accommodation for cattle became available.

VECTOR AND ARBOVIRUS ECOLOGY

The species of mosquitoes and Culicoides collected on and around the Peachester farm that yielded arboviruses over the course of the studies is shown in Table 1. Novel information on the potential vectors of a variety of arboviruses was assembled as a by-product of the search for the vectors of BEFV. A highlight was the isolation of Akabane virus from C. wadai, a Culicoides species that had recently arrived from northern Australia. The mosquito Cx. edwardsi yielded Sindbis, Corriparta group, and Mapputta group, Oak Vale plus an unidentified isolate.
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<th>Virus</th>
<th>Isolated From:</th>
<th>Number of Isolates</th>
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<td>Akabane</td>
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<td>31</td>
</tr>
<tr>
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<td><em>C. brevitaris</em></td>
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<td>Douglas</td>
<td><em>C. brevitaris</em></td>
<td>2</td>
</tr>
<tr>
<td>Akabane + Aino</td>
<td>Cattle</td>
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</tr>
<tr>
<td>Akabane + Tinaroo</td>
<td><em>C. brevitaris</em></td>
<td>1 (dual isolates)</td>
</tr>
<tr>
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<tr>
<td>Kimberley</td>
<td>Cattle</td>
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<td><em>C. brevitaris</em></td>
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<tr>
<td>Oak Vale</td>
<td><em>Culex sp.</em></td>
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<td><em>Cx. edwardsi</em></td>
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<tr>
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<tr>
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<td>Cattle</td>
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<td>Corriparta group</td>
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<td>Flavivirus group</td>
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<td><em>Cx. bitaeniorhynchus</em></td>
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<tr>
<td>Sindbis</td>
<td><em>Cx. edwardsi</em></td>
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<td><em>Ae(Verralina)sp.52Marks</em></td>
<td>4</td>
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<tr>
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<td><em>Cx. orbostiensis</em></td>
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<tr>
<td>Unknown/Unidentified</td>
<td><em>Ae(Verralina) sp52Marks</em></td>
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</tbody>
</table>
Kimberley virus

This virus has approximately the same distribution as BEFV. It was used retrospectively to discover the interaction with BEFV. Infections seemed to be more sporadic than forming an epidemic wave in the Peachester herd. This virus has approximately the same distribution in Australia and the world as BEFV.

Tibrogargan Virus

Nine isolates of this rhabdovirus virus were isolated from pools of *C. brevitarsis* collected between 24 November and 8 December 1976 on the Peachester farm in the Akabane study. Serological surveys showed that antibodies to Peaton virus were present in cattle distributed across northern and eastern Australia and in Papua-New Guinea (*Cybinski et al* 1980). This virus infected cattle in December-January in 1976 and 1977.

Oak Vale virus

The first isolation of another new rhabdovirus (CSIRO 1342) was made from *Culex edwardsii* mosquitoes collected on 30 December 1981. It was later named Oak Vale virus. No antibodies to this virus were found in cattle on the farm or elsewhere in Australia. Together with Tibrogargan Virus it was sequenced to form a large part of a PhD thesis, (Gubala 2009).

Akabane virus

Virus neutralization tests on the monthly bleeds of a sentinel group of young cattle on the Peachester farm had shown that Akabane virus infection occurred in the early part of the summer in 1974 and 1975. This meant that they had active antibody protection by the time they became pregnant and the foetus was spared infection and damage. There was no knowledge of how long the viraemia lasted. Akabane virus infection causes damage to foetuses through infection of the dam. It was presumed to be a silent infection. Accordingly a weekly program of blood sampling was initiated in August 1976 until 30 October 1976. Daily bleeds were made from 18-30 November. This was done in the anticipation that an infection with Akabane virus would occur. A matching insect collection for virus isolation was instituted from 24 November. The full viraemia period of three of the sentinels was captured and shown to be 3 or 4 days. No clinical effects were observed. *C. brevitarsis* was the dominant midge at the time and yielded 3 strains of Akabane virus. No viruses were isolated from other species of *Culicoides* or from mosquitoes. (*St.George et al* 1978). A dual infection of a bull at Peachester with Akabane and Aino viruses was proven by separating a double infection in a single blood sample from a bull. (*Cybinski and Zakrzewski* 1983).

Akabane virus was not transmitted transovarially through larvae collected from the Peachester farm in dung. The dung was collected at a time when Akabane was being isolated from female *C. brevitarsis* (*Allingham and Standfast* 1990).
Peaton Virus

The same study yielded Peaton virus as a new member of the Simbu group to which Akabane belongs. Nine isolates came from the same collections of *C. brevitarsis* as yielded Akabane virus. (St.George *et al* 1979, St. George *et al* 1980a).

Bluetongue virus

Selected serums from a Peachester cow where seroconversion to bluetongue group occurred were sent to England. The tests conducted there against the known serotypes of the world that identified the presence of BTV 1 in Australia. Thus the target was known and the relevant virus was isolated from a cow in the NT in 1979 (St.George *et al* 1980b). BTV1 was not isolated from *C. brevitarsis* collected on the Peachester farm until 14 February 1983.

D’Aguilar virus

An orbivirus (D’Aguilar virus) was isolated from blood samples collected from the blood of one of the sentinel group 9 times in a span of 11 days in the 1976 BEF epidemic (St.George and Dimmock 1976).

SENTINEL HERD SCHEME

The Peachester herd became the 19th herd to join the sentinel herd scheme from 1974 to 1986. This Australia wide project followed the distribution and seasonality of many arboviruses around Australia until CSIRO withdrew support in 1979 (St.George 1992). The historical serology for many arboviruses has been preserved in a national data bank. The Peachester farm is represented as a positive or negative spot on 20 virus or vector distribution maps. Unfortunately, the serum bank has been discarded. Otherwise other retrospective testing of the Peachester series would have yielded more to modern testing method that can detect antigens as well as antibodies.

MILK FEVER

Some very severe cases of BEF were treated with intravenous calcium borogluconate and phenylbutazone as described for experimental infections (Uren *et al* 1989). The production of lactating cows suffering BEF is reduced. Many cases of Milk Fever were also seen on the Peachester farm. Most were treated by the owner or one of the authors. In 1989, three cows and a heifer, expected to calve normally, were bled daily in the periparturient period for haematology and biochemistry. The object was to determine the haematology and biochemistry that had been applied in the study of BEF, differed from those of cows. At this stage, the dairy herd was being dispersed so only a few suitable animals were available. The three multiparous cows developed a relative neutrophilia in the periparturient period and the single heifer did not. (St.George *et al* 1995, 2001). If the idea had come years sooner the Peachester herd would have yielded some definitive results on comparison of BEF with milk fever.
DISCUSSION

The original objectives of the arbovirus and vector studies were achieved in regard to BEF. Natural BEF was followed on a daily basis during 4 epidemics. The haematology, biochemistry and interferon patterns were found to be similar in most aspects to the experimental disease. The severity of the inflammation was greater in natural disease as judged by the levels of fibrinogen found. The antibody response to infection with BEFV was complicated by pre-infection with KIMV. This vital data on the prior infection of cattle with KIMV and its effects on subsequent BEF could not have been found by any other means than the sentinel animal approach.

BEP passed through the Peachester herd in epidemic form in mid-summer in 1973, 1976, 1977, and 1984 and the cows that did not have BEF in one epidemic were infected in the next. There were no clinical cases between these waves. There were apparently no carriers and there was no overwintering by local vector species. The need to use a more specific test for BEFV antibodies was illustrated by the discovery of the heterotypic antibodies to BEFV so a specific test was developed by Zakrzewski et al (1992). BEFV was isolated from mosquitoes and Culicoides in association with the cattle. However, this did not occur until the isolation methods were improved toward the end of our study. The interaction of heterotypic antibodies and BEFV antigen before, during and after clinical signs was not fully appreciated until 1991. High fibrinogen levels in the clinical phase of BEF revealed the severe inflammatory nature of the disease for the first time. Young (1979) confirmed that fibrinogen levels were elevated in experimental cases though levels were not as high as were found in the Peachester herd. In effect this means that the inflammation was more severe in natural cases.

BEFV was isolated from C.brevitarsis and Anopheles bancrofti collected on the Peachester farm (Cybinski and Muller 1990). This seemed to indicate both were vectors although the distribution of C.brevitarsis was exceeded by the epidemic reach of BEF. The contradiction was not resolved until the experiments cited in St.George (2008) that C.brevitarsis could not be a vector because of its pool feeding method. The poor isolation rate of BEFV from Peachester cows in comparison to the success under experimental conditions is explained by the fact that antigen/antibody binding is limiting the availability of free virions in mouse or tissue culture inocula. This point is important in relation to the accumulation of BEFV strains to detect antigenic variability (Cybinski 1993).

The relationship between KIMV and BEFV infections was important in the understanding of the antibody responses in natural disease when compared to what was expected in experimental infections. KIMV is not known to produce clinical signs in experimental cases. However, only passaged virus was used in experimental infections. (MF Uren and SS Davis unpublished data) Experience has shown that passage of BEFV in mice or tissue cultures causes its attenuation for cattle. KIMV has approximately the same distribution as BEFV in Australia, and probably the world (Cybinski and Zakrzewski 1984). Preinfection with KIMV does not protect against infection with a subsequent BEFV infection. It is possible that KIMV was spread at the
same time as BEFV from an entry point in northern Australia after cattle arrived in northern Australia in the early part of the 19\textsuperscript{th} century.

Our appreciation of the differences between experimental EF and that studied in the field gained ground only slowly. The critical change came with the discovery and application of KIMV and its retrospective application to stored serum samples from this herd. The results showing cross-reactivity in natural infections with KIMV and BEFV guided Dr Cybinski to wider studies of the interactions of other members of the ephemerovirus group present in the Darwin area (Cybinski 1987). We were unaware that the more recently discovered viruses, Berrimah and Adelaide River, had reached Peachester before our studies there had concluded.

The steady flow of sentinel cow bloods to the laboratory allowed the course of many arboviruses through the sentinel groups to be followed as new arboviruses were isolated at Peachester or elsewhere. Almost all infections were subclinical. This is important in assessing the effects of arboviruses. The intensive studies involving daily bleeding yielded information on the length of detectable viraemias of several viruses. The total number of different arboviruses isolated from cattle or insects collected on this farm is 22. They are flavivirus group, Sindbis, Barmah Forest, BEFV, KIMV, Oakvale, Tibrogargan, Aino, Akabane, Douglas, Peaton and Tinaroo bluetongue1, Epizootic hemorrhagic disease virus serotypes 5,6 and 7, Bunyip Creek, CSIRO Village, D’Aguilar, Stretch Lagoon, Corriparta, Corriparta group and a Maputta group virus. In latter years of the investigation tissue cultures had completely replaced suckling mice as the method of arbovirus isolation at CSIRO Long Pocket Laboratories. Of the many arboviruses from the various families of arboviruses only BEFV, BTV, Aino and Akabane viruses are of known economic importance. However, if any others become associated with disease in animals their distribution and potential vectors are known. The Peachester farm yielded four arboviruses new to the world. These were Peaton virus, Stretch Lagoon, Tibrogargan and Oak Vale viruses. It is uncertain how many scientific publications have come from recent studies of these arboviruses.

All five of the known Simbu group viruses active in sentinel cattle were present in an 11 day time frame. (St.George 1985, Cybinski and Muller 1990). Akabane and Aino viruses were present in a single blood sample (Cybinski and Zakrzewski 1983). There is ample opportunity for Simbu group viruses to recombine, if this can occur. The sparing of some susceptible cattle in an epidemic situation was explained by the interference by an unrelated orbivirus CSIRO Village virus (St.George 1985). This could happen with other arboviruses of the same family.

The distance of the farm from Brisbane was a handicap for daily visits. Insect collections were transferred to the laboratory each second or third day. However, the rural area was free of city light effects. Small power torch bulbs in light traps attracted a range of species, Truck traps and animal bait traps were more effective with species not responding as well to light. The seasonality of arbovirus infections and serology correlated well with the presence of potential vectors between December and April.
(Cybinski and Muller 1990). The value of the continuous surveillance of a wide range of arboviruses in an ordinary dairy herd could not have been predicted. Clinical observations, virus isolation from cattle and potential vectors and serology were coordinated with entomology on a dairy herd typical of the region.

ACKNOWLEDGEMENTS

The immense generosity of the Hunt family in making their cows and their time available over many years made it possible to obtain information on arbovirus disease that could not be done in the laboratory. Harry Standfast was the mosquito expert always and the designer of improved insect traps. Gerry Murphy continuously improved and extended the biochemistry of natural disease as improved technologies became available. The material in this review was derived from published papers and the recollections of the authors from their input while members of the CSIRO Division of Animal Heath. Besides the authors, the main technical assistants were Judith Sack, Susan Moore, Trevor Daniel and Peter Allingham.
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