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Bluetongue and Related Viruses in New South Wales: Isolations from, and Serological Tests on Samples from Sentinel Cattle

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Abstract

Sentinel cattle at a number of localities in northern and central coastal New South Wales were sampled over the summer and autumn seasons of the years 1979, 1980 and 1981.

A total of 118 orbiviruses were isolated; 99 were of the Palyam group, 15 were of the epizootic haemorrhagic disease (EHD) of deer group, and 4 of the bluetongue group. The Palyam group viruses were identified by serotype as 68 Bunyip Creek, 23 CSIRO Village, 7 D'Aguilar and one was not typed. The EHD viruses were identified as 13 type 5 and 2 type 6. All 4 bluetongue viruses were type 21. There was also convincing serological evidence that bluetongue type 1 infection occurred in 1980.

Antibody to the bluetongue group, as demonstrated in a gel diffusion precipitin test, was often transient. It appeared to be mostly cross-reactive with, and induced by, other orbivirus infections, particularly those of the EHD group. Viruses of the Palyam group also seemed to be implicated in some circumstances. Where infections by viruses of the bluetongue group were demonstrated, the precipitating antibody responses to a bluetongue group antigen were not noticeably stronger than many which followed EHD virus infection.

The results generally confirm previous conclusions, deduced from serological surveys, regarding the frequency of orbivirus infections, the presence of bluetongue viruses, and the transient nature of many bluetongue group antibody reactions.

Introduction

Antibody that reacts with an antigen of bluetongue (BLU) virus type 20, and is detected in a gel diffusion precipitin (GDP) test (Sharp *et al.* 1988), occurs in cattle in New South Wales (NSW). Its geographic distribution and annual variations in its prevalence and distribution have been described by Burton and Littlejohns (1988) and Littlejohns and Burton (1988). GDP tests for BLU have generally been considered to be group-specific (Klontz *et al.* 1963; Jochim 1976) and the antigen used in the work reported here was shown by Sharp *et al.* (1988) to be shared by two other Australian BLU virus serotypes, but not by three members of the Palyam (PAL) group. The antibody reacting with it has therefore been held to be BLU group-specific, at least in regard to those Australian isolates.

To determine the origin and significance of the BLU group antibody found in NSW, blood samples were collected, for virus isolation and serological surveillance, from sentinel animals in the years 1979, 1980 and 1981.

Virus isolations are compared directly with the development of antibody in the same animals. Those of 1979 and 1980 are also compared with the fluctuations in prevalence of BLU group antibody found in wider serological surveys of cattle which were also conducted in those years (Littlejohns and Burton 1988).

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Abbreviations Used

BC, Bunyip Creek serotype of the Palyam group. **BLU**, bluetongue group, including serotypes 1, 20 and 21 (Parsonson and Snowdon 1985). **CSV**, CSIRO Village serotype of the Palyam group. **DAG**, D'Aguilar serotype of the Palyam group. **EHD**, epizootic haemorrhagic disease of deer group, including serotypes 5 and 6 (Campbell and St George 1986). **GDP**, gel diffusion precipitin. 'Strong' includes reaction strengths > or = 2, 'weak' = 1 (Littlejohns 1981). **NI**, neutralizing index. **VN**, virus neutralization. **PAL**, Palyam group, including serotypes BC, CSV and DAG (Knudson *et al.* 1984).

Table 1. Locations of herds and descriptions of sentinel groups sampled in the years 1979 to 1981

Location given as: latitude, longitude, pastures protection district (see fig. 1 of Burton and Littlejohns 1988)

Herd	Location	Number of animals/age		
		1979	1980	1981
I	28°49′ 153°26′ Tweed-Lismore	10/2–3 years	18/3–4 years[A]	9/adults
II	29°00′ 153°17′ Casino	10/1–2 years	4/yearlings 6/2 years 10/2–3 years[B]	10/2–8 months 10/2–3 years[B]
III	29°38′ 152°56′ Grafton	10/1–3 months	9/3 months 1/10 months 9/yearlings[B]	10/calves 10/yearlings[B]
IV	31°05′ 152°48′ Port Macquarie	10/3–7 months	10/6–10 months	10/9–12 months
V	32°34′ 152°08′ Maitland	–	10/8–12 months	10/4–6 months
VI	32°37′ 151°47′ Maitland	10/adults	10/adults	–
VII	32°49′ 151°51′ Maitland	10/5–7 months	–	–
VIII	32°37′ 151°35′ Maitland	10/4–6 months	10/5–7 months	10/4–6 months
IX	32°36′ 151°12′ Denman-Singleton	10/adults	–	–
X	32°50′ 151°45′ Maitland	–	10/5–7 months	–
XI	33°58′ 150°53′ Moss Vale	–	–	10/adults

[A] Includes eight from 1979. [B] Animals from the previous year.

Materials and Methods

Procedures, viruses and tests used evolved and expanded as information and materials became available, either from this work or otherwise, over the 3-year period.

Sentinel Stock and Sampling Procedure

Only animals that were negative in the GDP test for BLU at the start of each season were used as sentinels.

The locations of the sentinel herds, their inclusion in each year of sampling, and the number and age of stock utilized, are shown in Table 1. Samples were generally taken at weekly intervals over the summer and autumn of each year. Departures from this schedule are noted with results when relevant.

Clotted and unclotted (EDTA or heparin) 10 ml blood samples were taken for antibody tests and virus isolation respectively. Samples were cooled and transported in insulated containers. Transit time was usually 1–4 days, but on occasions was up to 10 days, a delay which did not prevent virus isolation from at least one sample. On another occasion Bunyip Creek (BC) virus was recovered from 2 of 20 samples which were 4 days in transit under heatwave conditions.

Virus Isolation

In 1979, virus isolation was attempted from the top 1 ml of cells of each sample after centrifugation. In 1980, the procedure was modified by washing the collected cells once in 10 ml of 0·9% saline and, after centrifugation, resuspending the packed cells in 1 ml phosphate-buffered saline. In 1981, the procedure was further modified by lysing the washed cells by the addition of up to nine volumes of sterile distilled water.

Prepared samples were inoculated to tube cultures of BHK21 cells which were maintained rolling and observed for cytopathic effects over 7 days. One further similar pass was made from material which was negative on the first.

In 1981, selected samples were inoculated intravenously to 10–12 day old embryonated eggs. The samples selected were usually those taken at, or the week before, the time when BLU group antibody was first detected in the donor animal. Embryos dying within 2 days were discarded. Subsequent deaths and survivors to 7 days post-inoculation were individually homogenized in phosphate-buffered saline, containing 1% gelatin plus antibiotics, and passed to BHK21 cells in the same manner as were original prepared blood samples.

Virus Identification

The first isolates of serotypes BC, EHD-5, EHD-6 and BLU-21 were plaque-purified before being identified by D. H. Cybinski of CSIRO Long Pocket Laboratories, Indooroopilly (personal communication). Other isolates were identified without purification. Preliminary identification was made by preparing virus group antigen by the standard method described for that of BLU-20 (Littlejohns 1981), and examining it in GDP tests for BLU, EHD and PAL groups. Final identification was made by virus neutralization, using the constant serum–varying virus method to determine neutralizing indices (NI). Reference neutralizing sera were used at dilutions which had NI of 2 $\log_{10}$ against the homologous virus. These sera were prepared in rabbits or, in the case of that against EHD-5, bovine antiserum was provided by T. D. St George of CSIRO Long Pocket Laboratories, Indooroopilly.

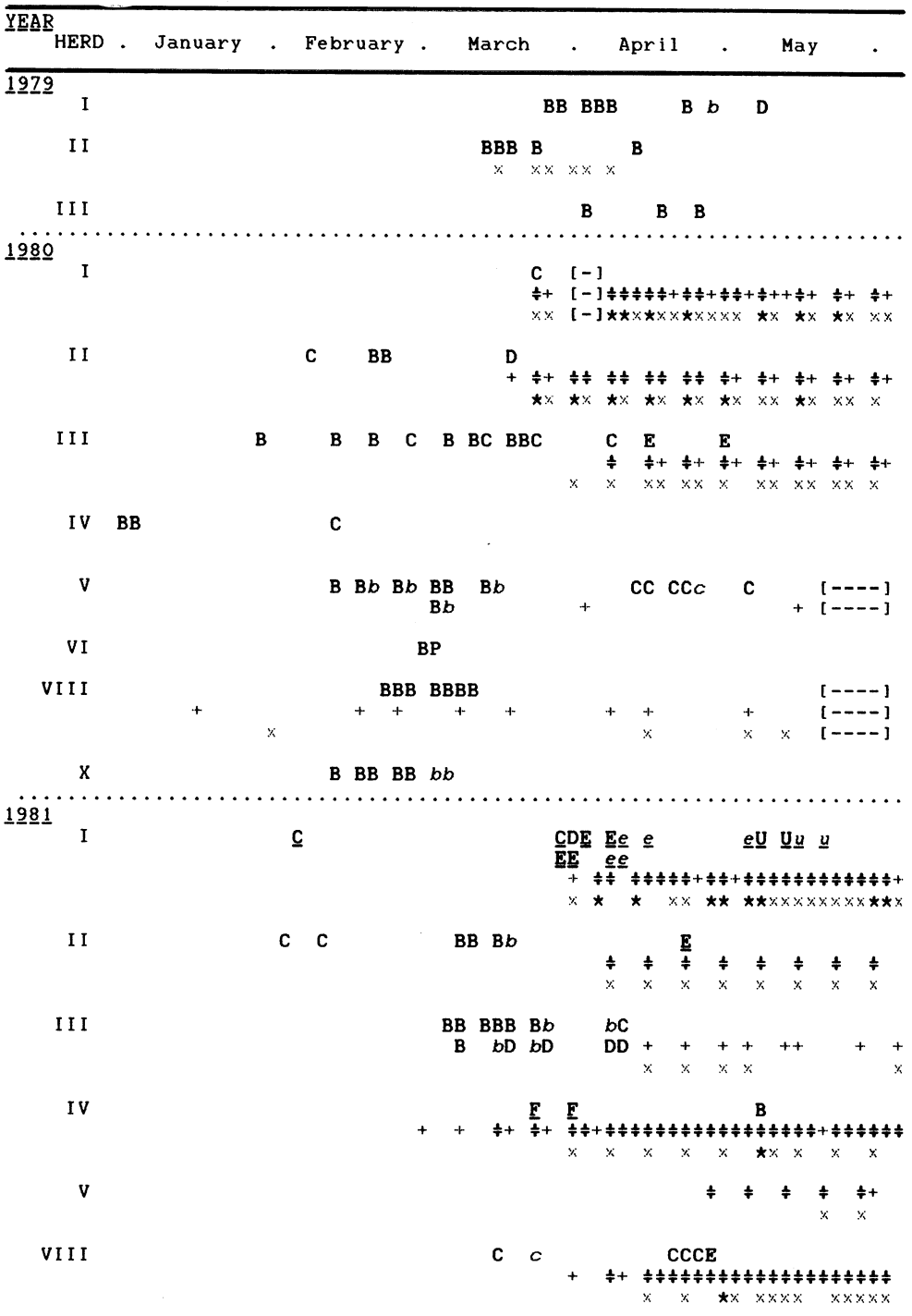
Tests on Sera

Reference materials were prepared, and GDP tests conducted, for antibodies to BLU, EHD and PAL virus groups using the methods described by Sharp *et al.* (1988), except that sera collected in 1979 were not tested for EHD. Virus neutralization (VN) tests were conducted by conventional methods in microtitre plates, using nominal virus doses of 100 $TCID_{50}$ and serial 3-fold dilutions of serum. Viruses used in VN tests were CSIRO 11 (representing CSV), B8112 (DAG) (Knudson *et al.* 1984); CSIRO 157 (EHD-5) (Campbell and St George 1986); CSIRO 156 (BLU-1), CSIRO 19 (BLU-20), CSIRO 154 (BLU-21) (Parsonson and Snowdon 1985) and local isolates, recovered in this work, representing BC and EHD-6.

Results

All virus isolations and herd summaries of BLU and EHD GDP tests are set out by date in Table 2 and only a general description of results for each year is given here.

Table 2. Virus isolations, BLU and EHD GDP antibody



PAL GDP results are not included, as reactions developed consistently according to PAL group virus isolations. Details relating to each herd, groups within herds and individual animals, and including points of interest in regard to the results of PAL GDP tests, are included in an Accessory Publication held by the Journal*.

1979

In all, 16 orbiviruses were isolated from 15 animals, all in the three most northern herds. All belonged to the PAL group, 15 being BC and one being D'Aguilar (DAG).

Development of BLU-group antibody was confined almost entirely to one herd (II). This herd differed from others, in which similar virus activity was demonstrated, in that the sentinel animals had pre-existing PAL GDP and CSIRO Village (CSV) VN antibody. The BLU antibody found was weak and was not detectable for more than 3 weeks.

1980

The level of orbivirus activity was higher than in 1979. This was reflected in both the range and distribution of viruses isolated and in the occurrence of BLU GDP antibody.

A total of 52 PAL (37 BC, 13 CSV, one DAG and one not typed) and 2 EHD (type 5) viruses were recovered.

Substantial amounts of BLU GDP antibody were developed in the three most northern herds. All samples from herds I and II which reacted in the BLU test were examined for VN antibody to BLU-1 and BLU-21, and one animal in each herd developed a high titre to BLU-1.

There were striking differences between different age groups, in both herd II and herd III, in pre-season antibodies, virus isolations and subsequent serological responses. For example, all 11 PAL virus isolations from herd III were from calves, none from yearlings, although most of the EHD and BLU GDP antibody subsequently demonstrated was found in yearlings.

PAL virus activity in herd V occurred in two distinct waves, one of BC only and one of CSV only.

1981

A total of 31 PAL (16 BC, 10 CSV and 5 DAG), 13 EHD (11 type 5 and 2 type 6) and 4 BLU (type 21) viruses was recovered.

The number of isolations obtained directly through cell culture (29 PAL and one EHD) was lower than in 1980 (52 PAL and 2 EHD), but a further 18 viruses (2 PAL, 12 EHD and 4 BLU) were obtained via embryonated egg passage.

*Available from the Managing Editor, Aust. J. Biol. Sci., 314 Albert Street, East Melbourne, Victoria 3002.

Legends to Table 2

B = BC virus isolated.
C = CSV virus isolated.
D = DAG virus isolated.
P = PAL-group virus, not typed.
E = EHD-5 virus isolated.
F = EHD-6 virus isolated.
U = BLU-21 virus isolated.

(Shown in upper case when this virus was first recovered, and lower *italic* when subsequently recovered, from an individual.)

(Underlined when recovered only by passage through embryonated eggs.)

ǂ = strong EHD GDP antibody.
\+ = weak EHD GDP antibody.
★ = strong BLU GDP antibody.
x = weak BLU GDP antibody.

(Each weekly sampling is represented by three ciphers. Each cipher represents up to one third of the group.)

[-] = No samples collected.

In VN tests for BLU-1 and -21, which were conducted on all BLU GDP test reacting sera, all were negative to BLU-1 and positive reactions to BLU-21 were found only in sera of the two animals of herd I from which that virus had been recovered. In those two animals, although VN antibody was first demonstrable 4 and 3 weeks after the first virus isolation from the respective animals, BLU GDP antibody developed concurrently with, or preceded by 2 weeks, the respective virus isolations. BLU GDP antibody in those two animals was not recognizably stronger or more persistent than that found in herd mates, with all of whom they shared serological evidence, confirmed by virus isolation from four, of near-contemporaneous EHD-5 infection. Surprisingly, there were no subsequent increases in BLU GDP antibody attributable to the BLU-21 infections.

Results tabled for herd II refer to calf sentinels only. There was very little serological evidence of any virus activity in adults, and no virus were recovered from them.

In herd III, the two animals from which EHD-5 virus had been isolated in 1980 were free of GDP antibody for BLU and EHD at the start of the 1981 season.

Although EHD-6 virus was recovered from only two animals in herd IV, EHD GDP antibody developed in all 10 sentinels. It was present in the two donors for 1 and 2 weeks, respectively, prior to virus isolation. EHD VN antibody also developed in all to one or both of EHD-5 and EHD-6 within 2 weeks either side of the EHD-6 virus isolations. Two animals remained negative to one serotype, one to each, confirming the specificity of the tests in this situation, and so the results are taken to indicate that both viruses had been highly active within the same time frame of 2 or 3 weeks.

No viruses were recovered from herd V, from which 17 isolations had been made in 1980, even though sentinels consisted of new young animals without demonstrable pre-season antibodies. GDP results were unusual in that only two developed reactions to PAL but all did to EHD. A brief period (3 samples, 2 weeks) of reactivity to BLU was detected in one of the two with both PAL and EHD antibody. It seems likely that failure to isolate the responsible virus(es) may be because material from the herd was not inoculated to embryonated eggs.

In contrast to the experience with herd IV, where there appeared to be no cross-reaction in VN tests for EHD-5 and EHD-6, in the different circumstances of herd VIII a low level of cross-reactivity, in one direction at least, was apparent.

In herd VIII, the development of BLU GDP antibody appeared to be related to PAL virus activity in animals with previous recent experience of an EHD virus.

Discussion

The range of orbiviruses known to infect cattle in New South Wales has been extended by the addition of two PAL serotypes, DAG and CSV; two EHD serotypes, 5 and 6; and one member of the BLU group, BLU-21. Convincing serological evidence that BLU-1 infections occurred in 1980 was also found. These findings confirm previous conclusions, based on single-bleed surveys (Burton and Littlejohns 1988; Littlejohns and Burton 1988), that both BLU-1 and BLU-21 had infected cattle in this State. The presence of viruses of the EHD group had not previously been suspected but, from the results of this work, they may be more commonly responsible for inducing antibody reactive in the BLU GDP test than are those of the BLU group itself.

Serological and virus isolation results also give support for other conclusions drawn from the single-bleed surveys. There was little virus activity or serological development in 1979, while in 1980 reactivity in BLU tests was largely limited to locations north of the Hastings River, and many of the GDP reactions were transient.

Variables affecting the data are recognized. Predictable effects of age, with young sentinels being more susceptible due to lack of previous exposures, and older animals primed to be more serologically responsive, were evident. The use of embryonated eggs on selected samples in 1981 considerably improved virus isolation rates, particularly for EHD and BLU (see herd I, 1981). The fact that the southern limits of antibody distribution were noticeably

contracted in 1979 (Littlejohns and Burton 1988), and that the year was found to be one of relatively low virus activity, is consistent with the expectation that the geographic limits of serological activity will reflect the level and distribution of past virus activity. However, even when allowance is made for recognizable variables, it appears that comparable virus activities have occurred almost synchronously in widely separated areas, and there is generally little indication of epizootic progression from north to south. For example, herds II and X, although about 400 km apart, had BC virus infections occurring in both at about the same time in 1980. Again, in 1981, EHD-5 virus was found in herd VIII at about the same time that it was active in herds I and II, which are also about 400 km distant from VIII. Consistent with the concept of discontinuous incidence, variations from year to year in the prevalence of orbivirus infections, within locations, can be quite remarkable. For example, compare results for herd V in years 1980 and 1981. There is no reason to suspect that changes in virus isolation procedure could be responsible for this difference.

EHD and BLU virus infections occurred towards the end of the observation period in 1980 and 1981. The earliest (seasonal) evidence of EHD infection was in March (herd IV, 1981). In contrast, PAL virus activity occurred virtually throughout the observation periods. Perhaps this reflects higher vector efficiencies and/or adaptation to the bovine host to favour PAL compared to EHD or BLU viruses.

The period over which reactions persist, and estimates of the sensitivity and specificity of a test, important for health certification purposes, can only be valid for the location and class of stock observed. In the interpretation of the group GDP reactions, and particularly those for BLU, in the area studied, the picture is one of transiency and cross-reactivity. BLU and EHD GDP reactions did not persist into the following season in those animals which were resampled even though some of the EHD reactions had developed after known EHD virus infections which resulted in persistent VN antibody. Brief reference has previously been made to transient BLU GDP antibody being found in animals in California, Colorado and Sudan (Sellers 1985), and their occurrence in New South Wales was deduced previously (Littlejohns and Burton 1988), when the importance of the fact for the proper interpretation of the results of these tests was stressed. It is also noted that GDP antibody was commonly present for up to several weeks before the isolation of virus which appeared to be relevant. This was particularly so in the case of infections by viruses of the EHD group, whether the reaction appeared to be of a specific or cross-reactive (BLU) nature. We have no adequate explanation for this relationship.

All identifiable BLU infections were followed by BLU GDP reactions which persisted for the remainder of the season. Hence, the sensitivity of the BLU GDP test, for detecting past BLU infection, was not observed to be less than 1·0 over that period. The few animals involved were not sampled further so no assurance was obtained that the high sensitivity would be maintained over a longer period. Sensitivity in regard to detecting cross-reactive antibodies must vary widely according to the nature and number of the infections responsible. Similarly, the specificity will vary over a wide range, inversely with the prevalence of BLU-related viruses. It may be high in a population which has not been exposed to any orbiviruses, for example, 99·9% in calves (herds IV, VII and VIII in 1979), or lower (99%) in adults (herds VI and IX of the same year); and much lower, of the order of 95%, even in calves, where, in the apparent absence of BLU viruses, there is known exposure to other orbiviruses, e.g. herd VIII in 1980. Had adult sentinels not been selected as BLU GDP negative at the start of each season, the apparent test specificity would undoubtedly have been lower again.

A useful perspective on cross-reactivity is given if the scope of antigenic diversity beyond the dominant determinants of conventional taxa (group or type) is considered. All isolates within single serotypes are not necessarily identical in regard to all epitopes of those antigens that are relevant to either serotype (VN) or group (GDP). In regard to serotypes, it is noted that antibody responses to 2 EHD viruses appeared to be quite independent in herd IV in

1981, but not so in herd VIII. In regard to group characteristics, antibody responses to agents of the same group and type, clustered by herd and time, often differed in potency and persistence. Presumably, these differences might be matched by variations in cross-reactivity between strains within types or groups. It therefore seems that rigid classifications by serotype or group may not be entirely approriate or adequate bases for the consideration of cross-reactivities. There is, within those classifications, scope for variation in immune priming and cross-reactivities that are serologically important, and in protective effects that are epidemiologically effective, according to the more precise antigenic composition of each virus strain.

The importance of an EHD virus in inducing cross-reactivity in the BLU GDP test is seen in herd I, 1981. A strong association was also apparent epidemiologically. In 1980, the restriction of BLU GDP antibody development to the area north of the Hastings River, recorded here, and previously from different data (Littlejohns and Burton 1988), is matched by EHD virus and antibody being of similarly limited distribution. In 1981, when more EHD virus activity was recognized, and seen over a wider area, BLU GDP antibody development was similarly more widely distributed.

Although PAL virus infections seemed generally to contribute little to the BLU antibody picture, there were several instances to suggest otherwise. In herd II in 1979, pre-existing PAL GDP antibody was the recognizable difference between that herd, which had some weak and transient BLU reactions after a period of BC and DAG activity, and other herds with apparently similar exposure to PAL group viruses in 1979, but no BLU group antibody detected. In herd III in 1981, some BLU reactions, again weak and inconsistent, followed PAL virus activity which included all three serotypes. Finally, in herd VIII in 1981, after earlier EHD GDP antibody development and then PAL virus activity, BLU GDP antibody appeared in phase with PAL GDP antibody. These results suggest that, under field conditions, at least some strains of PAL group viruses will contribute to BLU group antibody production in cattle.

Observations of the type described inevitably lack the control and certainty of those made on experimental infections. They are equally necessary as it is unlikely that any experimental design will match the range and diversity of factors that are effective under natural conditions. In the field, all of the viruses encountered appear to be able to contribute in some degree, depending on relationship, number, prior exposures or other circumstances, to any or all of the group antibody responses. Because of the frequency with which they were found and strengths of associated serological responses, viruses of the EHD group, and, to a lesser extent, those of the PAL group, appeared to be responsible for most of the BLU GDP antibody detected.

None of the viruses encountered was recognized to have had a pathogenic role.

Some of the conclusions discussed, together with notes on competition between viruses, virus isolation efficiencies and deductions regarding the course of natural infections, depend in part on detailed results which are contained in the accessory publication. They are presented and discussed more fully in that paper, as are cautionary notes relating to limitations to the ambits of relevance of observations and conclusions.

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