Archives of Virology 78, 151—165 (1983)

Archives of Virology

Evidence for a Bovine Origin of the Polyomavirus Detected in Foetal Rhesus Monkey Kidney Cells, FRhK-4 and -6

By

J. V. Parry[1], M. H. Lucas[2], J. E. Richmond[1], and S. D. Gardner[1]

[1] Virus Reference Laboratory, Central Public Health Laboratory, Colindale Avenue, London,
[2] Central Veterinary Laboratory, New Haw, Weybridge, Surrey, United Kingdom

With 2 Figures

Accepted August 5, 1983

Summary

Rabbit antisera to the stump-tailed macaque polyomavirus (STMV) which had been shown by immunoelectron microscopy and indirect immunofluorescence to react with the polyomavirus found in FRhK-4 cells (FRKV), also gave precipitin lines in counter-immunoelectrophoresis (CIE) and double diffusion in gel (GD) when reacted with FRKV. The reactions in GD showed identity with that of a rabbit antiserum to FRKV.

Naturally occurring antibody to FRKV (anti-FRKV) was found by CIE in 48 per cent of 353 cattle, 1/106 pigs and 1/20 goats but not in any of 13 other species including 45 rhesus monkeys and 97 humans. Each of 9 anti-FRKV positive samples from cattle, the goat serum, but not the pig serum gave a line of identity with the rabbit antiserum to FRKV in GD against FRKV. Detection of anti-FRKV in colostrum deprived newborn calves and in commercial foetal calf sera (FBS) indicates that intra-uterine infection of cattle with FRKV may occur.

FRKV adapted readily to growth in secondary calf kidney cultures and grew more rapidly and to higher titres than in the FRhK-4 cultures.

We conclude that FRKV is probably another strain of STMV and that the natural hosts of these viruses are cattle and not primates. Evidence of intra-uterine infection of cattle implies that infectious FRKV may be present in some FBS and may thus have gained entry into various susceptible cell lines, particularly primate kidney.

Introduction

The discovery of an adventitious polyomavirus in the FRhK-4 cell line and subsequently in a similar cell line, FRhK-6 has been reported by us (6, 7). These

two cell lines are representatives of several developed by WALLACE and her colleagues (15). One of these cell lines, FRhK-6, is known to have been used in the development of a live attenuated hepatitis A vaccine (8).

Many of the known polyomaviruses of mammals were primarily, and in some cases, solely isolated from cell cultures derived from kidney tissue (Table 1). Evidence of natural infection with the isolated polyomaviruses has been demonstrated in the presumed host species by the presence of serum antibody to that virus, except for the stump-tailed macaque virus (STMV) (9) and HD virus (14), the latter present in a cell line, Vero, derived from African green monkey kidney. These two viruses have subsequently been shown to be indistinguishable (4).

Table 1. *Host, origin and evidence of host antibody for the known polyomaviruses*

Virus	Reported host	Original isolation from	Naturally occurring host antibody
polyoma	Mouse	Pooled tissues	Positive
K	Mouse	Pooled tissues	Positive
LHV	Hamster	Spleen/kidney	Not known
RKV	Rabbit	Cutaneous papilloma[a]	Positive
SPV	Pig	Kidney cultures	Positive
WRSV	Cattle	Kidney cultures	Not known
LPV	African green monkey	Lymphoblastoid culture	Positive
SA12	African green monkey[b]	Kidney cultures	Positive
SV40	Rhesus monkey	Kidney cultures	Positive
STMV	Stump-tailed macaque	Kidney cultures	Negative
BK	Man	Urine	Positive
JC	Man	Brain	Positive

[a] Isolated simultaneously with a papillomavirus in rabbit kidney cultures
[b] Chacma baboon is the principal host

Immunoelectron microscopy (IEM) and indirect immunofluorescence (IF) have shown that the FRhK-derived virus (FRKV) is identical or closely related to STMV and is not one of the polyomaviruses SV40, SA12, BK or JC (10). As reported here the presence of antibody to FRKV (anti-FRKV) was not demonstrable in rhesus monkeys. It therefore seems likely that FRKV may be a further strain of the STMV/HD type. On the basis of the STMV model the natural conclusion would be that FRKV is also of primate origin. However, the observation was made by electron microscopy (EM) that free polyomavirions prepared from clarified tissue culture fluid (TCF) removed from FRhK-4 cultures were coated with a material morphologically indistinguishable from antibody, whereas virions from disrupted washed FRhK-4 cells were not antibody coated (10). The observation of unidentified structures attached to polyomavirions prepared from TCF is not novel (9, 13) and has been associated specifically with the bovine serum in the TCF (13). However, the possibility that, in addition to these observations, a virus-antibody reaction might also be present appears not to have been investigated. Our findings (10) have led us to investigate the possibility that one of the reacting components of bovine sera is antibody and that FRKV may be of bovine origin (7).

A technique for detecting antibody which was less laborious than IEM, but still applicable to serum from any species was sought. As it was not possible to demonstrate haemagglutination by this polyomavirus (10), counter-immuno-electrophoresis (CIE) and double diffusion in gel (GD) were investigated and found to be satisfactory. The results of a search for the presence of naturally acquired anti-FRKV by CIE in a variety of species are presented in this paper.

The discovery and characterisation of FRKV may not only be of consequence for the experimental attenuated hepatitis A vaccine for which the virus had undergone several passages in FRhK-6 cultures (8); the results presented below lead to conclusions which have far wider implications in the field of vaccine development. In addition, the description of STMV as a virus of stump-tailed macaques and its congenital transmission (11) must now be reconsidered.

Materials and Methods

Cell Culture

Cell Cultivation

i) FRhK-4: These cultures had been obtained from Dr. B. Flehmig, Tübingen University, Germany at passage 76 and were used up to passage 94 for these investigations. The cells were grown at 37° C on Eagles minimum essential medium (MEM) containing 6 per cent foetal bovine serum (FBS). Confluent cultures were incubated at 33° C on MEM with 2 per cent FBS which was replenished at weekly intervals. Cell stripping was carried out using phosphate buffered saline A (PBS) containing trypsin and versene.

ii) Calf Kidney (CK): Primary cultures were treated in a similar way to the FRhK-4 cultures except that 10 per cent FBS was used in the growth medium. Secondary cultures were prepared in 250 ml flasks. One flask from each batch of secondary cultures was set aside for long-term incubation in an attempt to isolate a polyomavirus directly from calf kidney cell culture. The cultures were maintained for part of the time on serum-free medium, Iscoves modification of Dulbecco's MEM, complete medium (Flow Labs.) and part of the time on MEM with 2 per cent FBS. Preparations of low-speed pellets from the TCF collected from these cultures were negatively stained with 3 per cent phosphotungstic acid, pH 6.3, and examined by EM.

Virus Propagation

i) FRhK-4: Because many commercially available pooled bovine sera preparations appeared to possess antibody (10 and below), the chronically infected cultures were incubated with Iscoves medium. The confluent cultures were washed twice with PBS before receiving the Iscoves medium. The cultures were then incubated stationary at 37° C with medium changes at two week intervals.

ii) CK Cultures: Passage 1: Two flasks of confluent 2° CK were washed with PBS. One flask, the control, received 20 ml of Iscoves medium alone; the other received 20 ml of clarified Iscoves medium harvested from 2 cultures of FRhK-4 cells which had been seeded 12 weeks previously and had received their last medium change 2 weeks earlier. The CK cultures were incubated at 37° C and the medium was replenished at days 5 and 13. The TCF and cell sheets were harvested together at day 27.

Passage 2: Three flasks of confluent 2° CK were washed with PBS and then inoculated with 1.5 ml of frozen and thawed harvest from passage 1 at day 27. After adsorption for 3 hours at 37° C, 20 ml of fresh Iscoves medium was added to each and incubation continued for 17 days at 33° C. The medium was replenished at day 14. Uninoculated control flasks of the same 2° CK batch were incubated simultaneously.

Passage 3: Two flasks received 7.5 ml each of clarified TCF, pooled from days 14 and 17 of passage 2. After adsorption the inoculum was supplemented by 15 ml of Serumless Medium (Gibco) with 2 per cent gammaglobulin-free equine serum (EqS). The TCF was replenished at days 8 and 13 and the passage terminated at day 15.

Polyomavirus Antigen Preparation

a) Crude Preparation: It had been noted that the cells which are released into the TCF yielded most virus (10). Therefore, cells were pelleted from TCF by centrifugation at $1500 \times g$ for 10 minutes, resuspended in a small volume of water and sonicated in a bath sonicator to release the virus. The sonicates were used as FRKV antigen in some of the preliminary CIE tests.

b) Semi-purified Preparation: Sonicates of cell pellets were prepared as in a). Larger particulate material was removed by low-speed centrifugation and then 0.5 ml of each supernatant placed on a density gradient composed of 30, 40, 50 and 60 per cent weight/volume sucrose in distilled water which had been allowed to equilibrate. The gradients were centrifuged at $250{,}000 \times g$ for 4 hours in a swing-out rotor and then 14—18 fractions, each of 5 drops collected from each of them. The fractions were tested for FRKV by CIE (see below) and those giving a specific reaction pooled for use as antigen.

Counter-Immunoelectrophoresis (CIE)

The method used was that described by COHEN *et al.* (2). The polyomavirus antigen was used at 4—8 times its CIE end point. Newborn calf serum, NBS 22/6, which was positive for anti-FRKV by IEM, CIE and GD was designated the antibody positive control for CIE and was included at least once on every CIE plate used for antibody testing. NBS 22/6 was also used as the antiserum in CIE tests for the detection of FRKV antigen. Gels were examined for precipitin lines which were scored on a scale 0 to $++++$.

EM Examination of Precipitin Lines

A sample of precipitin lines was selected from the various groups of sera tested by CIE and examined by EM for virus-antibody reactions. The lines were carefully cut out, homogenised in 20—40 µl of water, applied to formvar/carbon-coated grids and negatively stained as above.

Double-Diffusion in Gel

Wells (3 mm) were cut in a hexagonal pattern around a central well. The gel consisted of 0.75 per cent agarose in a barbitone buffer of which 3 ml had been poured into a 35 mm plastic petri dish. The virus preparation was placed in the central well and the sera in the outer wells. The wells were refilled after 4 hours and the gels examined for precipitin lines 24—48 hours later.

Receptor Destroying Enzyme (RDE) Treatment

Serum samples from cattle were mixed with RDE (Wellcome Reagents Ltd.) in the proportion of 1 part to 4 parts, incubated at 37° C overnight and then inactivated at 56° C for 1 hour. The experiment was controlled by treating duplicate samples similarly except that saline was substituted for RDE. Both samples were then tested for anti-FRKV by CIE.

Immunoglobulin G (IgG) Purification

Immunoglobulin was precipitated by ammonium sulphate from two cattle sera that contained anti-FRKV. The immunoglobulin from one sample, NBS 22/6, was then further purified both by affinity chromatography, utilising protein A-sepharose and by an ion exchange technique using DE 52 (Whatman) under IgG eluting conditions of 50 mM phosphate buffer, pH 6.3. The second serum, BW 14467, was not processed by the protein A technique. The protein concentrations of the purified IgG were determined by optical density measurement of the samples at 280 nm and then adjusted to approximately 10 mg/ml.

Results

Virus Propagation and Antigen Production

FRhK-4 Cultures

Sufficient quantities of FRKV antigen for use in CIE were not detected until at least 6 weeks after seeding the FRhK-4 cultures. Table 2a shows the antigen titres in CIE of a series of different cell culture preparations. Although 4 preparations, 1, 3, 5 and 8 were sufficiently potent for CIE, two of these, 5 and 8, were of poor quality, and gave rise to indistinct non-specific precipitation which masked the sharp specific lines of the virus-antibody reaction. These non-specific reactions increased as the antigen aged and were soluble upon soaking the CIE gel in PBS, whereas the specific precipitin lines were unaffected. The FRKV antigen preparations which were unsuitable for direct use in CIE were combined (FRhK-4 Pool) before concentration and purification.

Table 2. *Reactions of crude FRKV antigen preparations with anti-FRKV in CIE*

a) *FRhK-4 cultures*

FRKV antigen preparation	Results of antigen titration in CIE versus NBS 22/6 (neat)						Application
	Undil.	1/2	1/4	1/8	1/16	1/32	
1	###	##	+	±	—	—	CIE
2	##	#	±	—	—	—	FRhK-4 pool
3	###	##	#	+	±	—	CIE
4	#	+	—	—	—	—	FRhK-4 pool
5	##	#	+	±	—	—	FRhK-4 pool
6	##	±	—	—	—	—	FRhK-4 pool
7	##	±	—	—	—	—	FRhK-4 pool
8	##	#	+	±	—	—	FRhK-4 pool
b) *Secondary calf kidney*							Comments
CK 1/CON	—	—	—	—	—	—	uninoculated control day 27
CK 1/FRKV	##	++	+	±	±	—	FRKV passage 1 in CK, day 27
CK 2/FRKV	##	##	##	##	#	+	FRKV passage 2 in CK, day 17

Purification of the FRKV antigen on SDG separated the material which causes the non-specific precipitation; this remained near the top of the gradient, whereas the viral antigen migrated through about two-thirds of the gradient (Table 3a). The pooled CIE positive fractions had undergone approximately 16-fold concentration by volume from the starting material, the FRhK-4 Pool, to yield a sufficiently potent CIE antigen (Table 3b). Non-specific activity could not be detected by CIE in the SDG purified antigen.

Table 3. *Semipurification of FRKV from cell homogenates of FRhK-4 and CK cultures*

a) *CIE test results on sucrose density gradient (SDG) fractions*

	Gradient fraction													
	Heavy →													Light
Sample	1	2	3	4	5	6	7	8	9	10	11	12	13	14
FRhK-4 pool	−	−	+	++	+++	+++	++	+	−	−	−	±	+	+++
CK 2/FRKV	−	−	+	+++	+++	+++	++	+	−	−	±	±	±	++
CK 1/control	−	−	−	−	−	−	−	−	±	±	±	±	−	−

b) *CIE titre of SDG — purified FRKV pools*

	Results of antigen Titration in CIE versus NBS 22/6 (neat)					
Sample	Undil.	1/2	1/4	1/8	1/16	1/32
CK 1/control (1×)	−	−	−	−	−	−
CK 1/CK 2 pool FRKV (1×)	+++	++	+	±	−	−
FRhK-4 pool (16×)	+++	++	+	±	−	−

Calf Kidney Cultures

FRKV was found to adapt readily to calf kidney cultures. Widespread involvement of the cell sheet occurred in successively shorter periods at each of the first 3 passages; 27 days, 17 days and 15 days. In addition, areas of the typical polyomavirus cytopathic effect (CPE) of cytoplasmic vacuolation became more extensive at each passage. The uninoculated control cultures remained unchanged. Fig. 1 shows the appearance of the CPE in a CK culture on day 17 of the third passage of FRKV in CK cultures and that of the control culture. The yield of viral antigen by CIE is given in Table 2b for passages 1 and 2 and a control culture. These results confirmed the impression given by microscopic examination of cultures for CPE.

The crude FRKV antigen from CK cultures was of higher titre and did not produce the non-specific reactions shown by the FRhK-4 product. Purification by SDG was however carried out. Banding of the viral antigen in the gradient was similar to the FRhK-4 derived antigen (Table 3a) and the pooled SDG fractions gave an identical potency without the necessity of concentration (Table 3b). The control preparation showed traces of non-specific precipitation near the top of the gradient.

Examination by EM of disrupted cell pellets from the TCF of 7 different batches of 2° CK which are on long-term incubation has failed to reveal the presence of polyomavirions. The cultures had been maintained for between 16 and 69 days since seeding.

Identity of FRKV

FRKV gave specific precipitin lines when tested in CIE against two rabbit antisera to the polyomavirus STMV, kindly donated by Dr. K. V. Shah, Johns

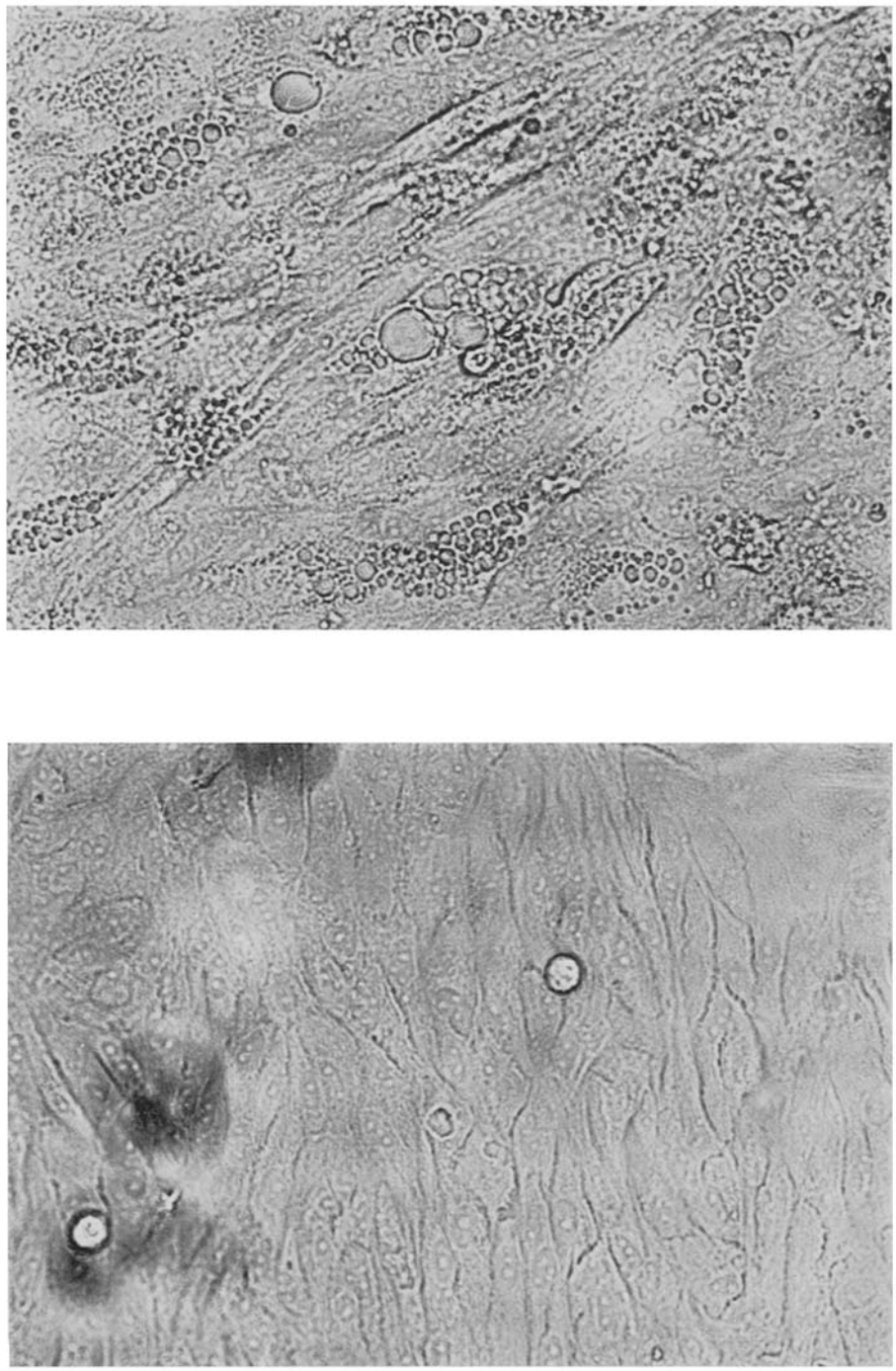

Fig. 1. Characteristic polyomavirus cytopathic effect caused by FRKV infection of secondary calf kidney cells after 15 days incubation (top) and the uninfected control cells (bottom). $\times 330$

Hopkins University, Baltimore, U.S.A., and against a rabbit antiserum to FRKV prepared in this laboratory. Antisera against polyomaviruses BK and JC, which gave precipitin lines against their homologous antigen, did not precipitate FRKV in CIE.

When STMV and FRKV antisera were tested in adjacent wells of a double diffusion in gel test against the purified FRKV antigen, a line of identity resulted. Such a line of identity was observed with each of the STMV rabbit antisera, 75—176 and 81—239. It was not possible to perform this test against the STMV antigen as this was not available.

Verification of a Virus-Antibody Reaction

The three investigations carried out to confirm that the virus precipitation detected by CIE was mediated by antibody gave results which were compatible with this type of reaction. Firstly, immunoglobulin G which was purified from cattle sera by the standard techniques outlined above continued to precipitate FRKV. Secondly, identical reactions were obtained from serum samples which were RDE treated to remove any non-specific inhibitors and from the same samples which received the control treatment. Finally, EM examination for polyomavirions coated with antibody in the CIE precipitin lines formed between the FRKV antigen and the following bovine samples confirmed a virus-antibody reaction: 2 NBS, 5 individual bovine sera, 2 IgG preparations purified from NBS, 2 RDE treated NBS and 1 FBS. Two additional FBS samples gave rise to equivocal EM results since only small amounts of antibody were observed attached to the virions.

Serological Surveys by CIE

Screening of Various Animal Species for Anti-FRKV

Only a few of the species listed in Table 4 were found to possess antibody to FRKV. Forty-eight per cent of sera from cattle collected from a number of herds were found to be positive. The one goat found to be seropositive was bled at the same time as 3 others from the same herd which were negative. The pig which was positive was the only one out of 7 adults from the same herd. Anti-FRKV was not found in any primates examined, including 45 rhesus monkeys and 97 human blood donors.

Detailed Analysis of CIE Results on Cattle Sera

The distribution of anti-FRKV in cattle relative to their age is shown in Fig. 2. Forty per cent of newborn calves were seropositive. At 12 months only 11 per cent were definitely positive and thereafter an upward trend towards a very high proportion of seropositives was noted. Of the 273 cattle sera represented in Fig. 2, 230 were known to be from the females of 11 herds of Friesians or Holsteins in England and Wales. The proportion of anti-FRKV positive cattle for each herd varied between 24 and 75 per cent with a mean of 52 per cent. The prevalence of seropositivity seemed to bear no relationship to the area of the country in which the herd was kept.

Table 4. *Results of screening for the presence of anti-FRKV by CIE in 766 sera collected from 16 animal species*

Source of sera	No. tested	No. positive	No. equivocal
Fowl	5	—	—
Pigeon	5	—	—
Mouse	22	—	—
Rat	10	—	—
Rabbit	14	—	—
Hamster	7	—	—
Guinea pig	12	—	—
Pig	106	1	—
Sheep[a]	27	—	1
Goat	20	1	—
Cattle	353	170	12
Marmoset	12	—	—
Baboon	25	—	—
Rhesus monkey[b]	45	—	—
Assorted primates	6	—	—
Human (blood donor)	97	—	—

[a] 7 of these animals had been used to prepare hyperimmune sera to echoviruses
[b] One animal immunised against malaria

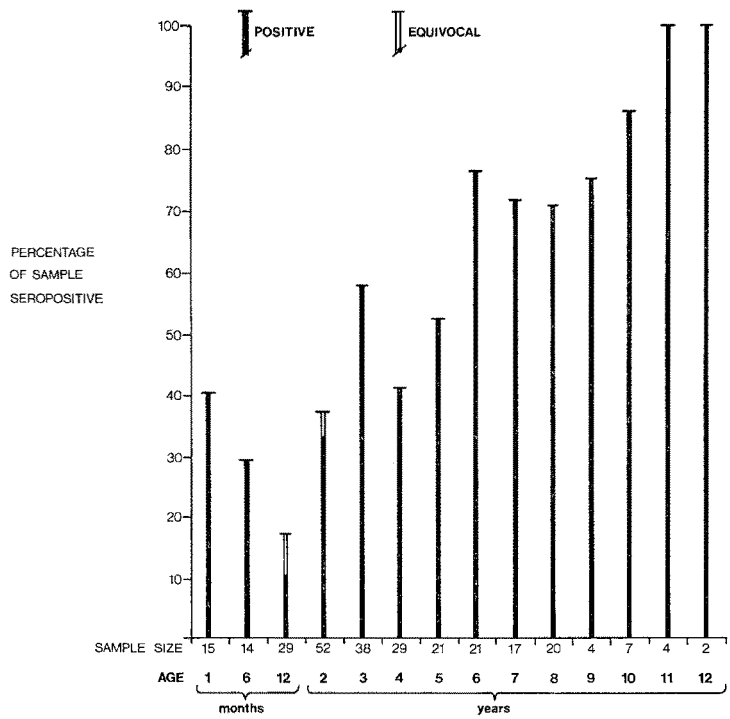

Fig. 2. Age distribution of anti-FRKV, detected by CIE, in 273 cattle

Commercially Available Sera

Thirty-five different batches of cattle serum pools, marketed for tissue culture work, were obtained from several commercial sources and tested for anti-FRKV by CIE. The results are shown in Table 5. Overall, 69 per cent of these pooled sera contained anti-FRKV, but whereas all of the newborn calf sera contained anti-FRKV, only 59 per cent of the foetal calf sera were definitely positive and 22 per cent did not have detectable levels of anti-FRKV when tested by CIE.

Table 5. *Results of testing 35 commercially produced cattle serum pools for presence of anti-FRKV by CIE*

Serum	Positive	Equivocal	Negative	Total
Foetal calf	16 (59%)	5 (19)%	6 (22%)	27
Newborn calf	7 (100%)	—	—	7
Donor calf	1 (100%)	—	—	1
Totals	24 (69%)	5 (14%)	6 (17%)	35

Colostrum Deprived Calves

Thirteen sets of mothers and calves which were not included in the detailed analysis above (b) were tested for anti-FRKV. The calves were bled immediately after birth and were not permitted to suckle, in order to prevent transfer of maternal antibody in the colostrum. Only two sets of mother and calf, CD2 (CIE titres: neat and 1/16 respectively) and CD10 (CIE titres: both 1/8) had detectable levels of anti-FRKV in their serum. Serum from one additional mother, CD12, gave rise to a definite, but very faint precipitin line in CIE, which was not typical of the specific reaction. Colostrum was also available for 12 of the sets and these were tested for anti-FRKV. (Unfortunately colostrum was not available from CD10.) It was found that anti-FRKV was not only present in the colostrum of the seropositive mother, CD2 (CIE titre 1/32), but also in those of CD1, CD7, CD8, and CD11 (CIE titres: 1/8, 1/2, 1/4 and 1/8 respectively). Colostrum from CD3 reacted very weakly when tested undiluted. In mother CD2 the titre of anti-FRKV in the colostrum was approximately 32-fold higher than in her serum.

Double Diffusion Tests on Animal Sera

In order to investigate serological identity between naturally occurring anti-FRKV and that prepared in rabbits the following specimens were selected for GD tests as a representative sample of those that were reactive by CIE: two batches of commercial newborn calf serum, 2 sera from colostrum deprived newborn calves, 3 sera from adult cattle, 2 bovine colostrum samples, 1 goat and 1 pig serum.

The results fell into 3 categories. Ten samples gave a line of identity with the rabbit antiserum reaction, but these could be placed in 2 further sub-divisions: those which gave only a line of identity, of which there were 6 (5 bovine and 1 goat) and those samples (2 colostra and 2 bovine sera) which gave rise to other

lines in addition to the one continuous line which signifies identity: one gave rise to a spur, another to a second line of identity and two did not show identity of reaction. Finally, one sample, the pig serum failed to react with the FRKV antigen but a strong reaction occurred between the pig serum and both the pre-immune and immune rabbit sera which were in adjacent wells. The adjacent rabbit antiserum did continue to precipitate the FRKV antigen.

Discussion

Polyomaviruses are considered to be essentially host specific in their *in vitro* growth characteristics (5). The results presented here and elsewhere show that FRKV and STMV do not grow very readily in the primate kidney cultures in which they were discovered. FRKV was detected only after extended incubation of the FRhK-4 and FRhK-6 cell cultures (10). Similarly, SHAH *et al.* (11) were unable to find specific STMV IF until at least 8 weeks after initially preparing the kidney cultures, when it was demonstrated in tertiary cultures. Calf kidney cultures, however, proved to support replication of FRKV very well. Indirect immunofluorescence demonstrated that a much greater proportion of CK cells, 75 per cent, became infected with FRKV (10) than was the case for the FRhK-4 and stump-tailed macaque kidney cultures, in which only 5—10 per cent had specific nuclear fluorescence (7, 9). The FRKV inoculated CK cultures did not require lengthy incubation to produce an adequate antigen for CIE. This antigen may not have needed purification as it was little affected by the non-specific reactivity seen in the crude FRhK-4 derived antigen which required purification and concentration before use.

The serological results obtained by IEM and IF (10) and those of CIE and GD reported here indicate that, at very least, STMV and FRKV are closely related antigenically. As STMV was not available to carry out two-way matching, the possibility does exist of a one-way cross reaction, but none of the other polyomaviruses seems to possess such an inter-typic antigenic relationship. This leads us to conclude that STMV and FRKV, and consequently HD, are one and the same polyomavirus.

Because FRKV was isolated from rhesus monkey tissues it might have been concluded that the natural host of this virus is the rhesus monkey, in much the same way that STMV has been related to stump-tailed macaques. The failure to demonstrate anti-FRKV in rhesus monkeys parallels the failure to find anti-STMV in stump-tailed macaques in which this phenomenon has been explained using a hypothesis of vertical transmission and tolerance in the infected offspring (11). In this respect STMV, and therefore FRKV, would stand alone amongst the known polyomaviruses (Table 1). All kidney cultures prepared from 27 adult and foetal stump-tailed macaques yielded STMV (11) and so it has been postulated that STMV carriage is very common, if not universal in the stump-tailed macaque population (12), but the presence of STMV or its antigens has never been demonstrated *in vivo*. The hypothesis, however, is unlikely because stump-tailed macaques have been challenged with STMV and shown to mount an immune response indicating that these animals had not been rendered tolerant (12).

Evidence of natural exposure to FRKV was found almost entirely in cattle, of which a large proportion were found to be seropositive. FRKV therefore seems commonly to infect cattle, though nothing is known which connects the virus to any particular disease. The finding of anti-FRKV in 1/100 pigs or 1/20 goats might be explained if they had been in close proximity to cattle or to their body tissues or fluids. The relationship between age and seropositivity suggests that a proportion of newborn calves receive passive anti-FRKV from colostrum which becomes undetectable after several months, and that they subsequently become actively immune due to infection by horizontal transmission of FRKV. An alternative explanation is that the increase in anti-FRKV prevalence with increase in age is due to reactivation of a latent FRKV infection in at least some of the cows. This could then boost anti-FRKV from undetectable to detectable levels by CIE. Because the half-life of bovine IgG is about 3 weeks it is improbable that CIE is sufficiently sensitive to detect anti-FRKV which has been acquired passively, during the first day after birth, for a period of 9—12 months.

The detection of anti-FRKV in the colostrum of cows which were seronegative is not an anomaly; colostrum is known to contain several times the concentration of IgG found in serum and may therefore be positive when the serum appears to be negative, thus offering a more sensitive means of demonstrating previous exposure to the virus in pregnant cows near term. The high level of colostral IgG is attained by transfer from, and consequent depletion of, serum IgG in the pregnant cow. The maternal serum IgG levels return to normal within 24 hours after calving. A detectable CIE reaction from the serum of these cows may thus indicate a recent antigenic stimulus.

Reactivation during pregnancy has been demonstrated in the case of the human polyomaviruses, BK and JC (3), and this may also occur with bovine polyomavirus in some pregnant cattle. Because transfer of maternal antibody through the bovine placenta does not occur, the presence of anti-FRKV in the colostrum deprived calves may be interpreted as evidence that an intra-uterine infection with FRKV has occurred. This conclusion is supported by the observation that only the mothers of the 2 seropositive calves were seropositive although other mothers had anti-FRKV in their colostrum indicating previous experience of FRKV. The demonstration of serum anti-FRKV may indicate a recent infection or reactivation in these 2 cows, presumably during pregnancy, and consequent infection of their foetuses.

The finding of anti-FRKV in a substantial proportion of FBS batches has similar significance, indicating that some of the donations added to the pool must have been from foetuses which had been infected with FRKV. Because of the method of bleeding the foetuses (from the heart) it is most unlikely that contamination of the pool with sufficient seropositive maternal blood to cause positive results in CIE could occur.

If intra-uterine infections do occur then it is likely that some of the batches of FBS may have contained one or more donations carrying infectious FRKV. Although in some cases there may be sufficient anti-FRKV in the serum pool to cause neutralisation, some FBS pools do not contain detectable levels of anti-FRKV and these may occasionally contain infectious FRKV. Investigation of this may prove extremely difficult because there could be too few virus particles

present to be detectable by direct means and because most cell cultures have been, and are, grown on medium containing FBS and therefore semi-permissive cells may already be infected with FRKV.

Attempts to show complete identity by GD between the FRKV antiserum raised by rabbit immunisation and the naturally occurring antibodies detected by CIE in farm animals were in some cases difficult to interpret because more than one precipitin line was present. This might be due to a difference in response between immunisation and natural infection. The natural response seems to be more complex and the additional precipitin lines in GD may be due to more than one class or sub-class of immunoglobulin. The results do indicate in some cases at least partial and in others complete identity of the antigenic stimulus given by the polyomavirus in cattle and that given by FRKV, and by inference STMV, in rabbits.

In 1980, COACKLEY *et al.* (1) reported the detection of a polyomavirus, which they called WRSV, in tissue cultures prepared from the kidneys of a healthy newborn colostrum deprived calf. The presence of the virus was discovered when eosinophilic intra-nuclear inclusions were observed in CK coverslip cultures. The virus was readily passaged in CK cells, but replication was not detected in several other cell culture systems. WRSV failed to agglutinate red blood cells from chickens, sheep, guinea pigs and man. We are planning to undertake serological tests with WRSV in order to clarify the relationship between STMV, FRKV and WRSV. It seems likely that this probable bovine polyomavirus, WRSV, is a naturally occurring strain of these viruses.

The evidence so far points to the conclusion that FRKV is very similar to the virus designated STMV. Serological data lead us to believe that cattle, not primates, are the natural hosts of FRKV and consequently STMV. On the basis of studies of colostrum deprived calves and FBS samples we would, in addition to horizontal transmission, propose that *in utero* transmission of FRKV due to either reactivation or primary infection occurs in a significant proportion of pregnant cattle. This would explain how primate tissue culture lines could become infected with a bovine polyomavirus since some batches of FBS may contain infectious FRKV. The infection may be non-cytopathic and therefore go unrecognised unless positive measures are taken to detect it.

Several questions remain unanswered: how widespread is the contamination of laboratory cell lines with this bovine polyomavirus, especially those being used in the field of vaccine development? How significant would the finding of this polyomavirus be in such cell lines? Is FRKV capable of infecting man and if so what might be the outcome? Although we have reported its ability to replicate in human embryonic kidney cells (7, 10), we have so far been unable to find evidence of anti-FRKV in human sera (Table 4).

Addendum

Subsequent to the preparation of this paper we have been able to carry out some preliminary serological tests on the bovine polyomavirus WRSV, kindly provided by Mr. W. Coackley, Animal Health Laboratory, S. Perth, Western

Australia. We have demonstrated that WRSV reacts by CIE with 2 rabbit antisera to STMV (75—176 and 81—239), the rabbit anti-FRKV and newborn calf serum, NBS 22/6. WRSV also gave specific nuclear fluorescence with rabbit anti-STMV (75—176) in IF. WRSV does therefore seem to be antigenically very similar to STMV and FRKV.

Acknowledgments

We are indebted to Mr. J. R. Lukey, C. V. L. Weybridge, for supplying the 1° calf kidney cultures, Mr. M. Dawson, Miss S. F. Cartwright and Mr. B. N. Parker also from C. V. L., for donating samples from sheep, goats, pigs and cattle, and to Mr. P. Gerson, C. P. H. L., for providing many of the blood samples from the laboratory animals. We would also like to thank Drs. M. S. Pereira and P.P. Mortimer for their critical reading of the manuscript and Miss B. Mandalia for typing of the tables.

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Authors' address: Mr. J. V. Parry, Virus Reference Laboratory, Central Public Health Laboratory, Colindale Avenue, London NW 9, United Kingdom.

Received July 5, 1983