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COMPARATIVE SUSCEPTIBILITY OF A CANINE CELL LINE AND BLUETONGUE VIRUS SUSCEPTIBLE CELL LINES TO A BLUETONGUE VIRUS ISOLATE PATHOGENIC FOR DOGS

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SUMMARY

Recently, bluetongue virus (BLU) serotype 11 was detected in diseased dogs that had been inoculated with live attenuated vaccine contaminated with this serotype of bluetongue virus (Akita et al., 1994). For various laboratory tests, BLU can be propagated in different cell cultures. No information was found in the literature about the possibility of propagating this virus in canine cells. To determine whether the BLU isolate from the contaminated canine vaccine (BLU-vac) is unique in its ability to replicate in canine cells, this virus was studied in parallel with U.S. prototype strains of BLU (serotypes 2, 10, 11, 13, and 17), in hamster lung (HmLu-1) and canine kidney (MDCK) cell cultures. In HmLu-1 cell cultures, the BLU-vac produced cytopathic effect (CPE) of the same type as the U.S. prototype BLU strains by 4 to 6 d postinoculation. In MDCK cell cultures, all of the BLU strains tested were able to replicate but did not produce CPE. The BLU-inoculated MDCK cells became persistently infected, and these cultures continued to produce infectious BLU even after six serial passages over 2½ mo. In none of these cultures was CPE observed. In mixed cultures containing both HmLu-1 and MDCK cells, CPE first affected the HmLu-1 islands; subsequently, CPE spread also to the areas with MDCK cells. The silent persistent infection of the MDCK cells with BLU indicates that more stringent screening of the cells used in the production of live vaccines for various contaminating viruses is necessary.

Key words: bluetongue virus; dog; cell culture; vaccine.

INTRODUCTION

Bluetongue virus (genus Orbivirus, family Reoviridae) has been considered to be pathogenic for wild and domestic ruminants only (Knudson and Monath, 1990; MacLachlan et al., 1991; Roy, 1991). Recently, however, bluetongue virus (BLU) serotype 11 was detected in diseased dogs that had been inoculated with a live attenuated polyvaccine (antidistemper, adeno 2, parainfluenza, and parvo viruses) contaminated with this serotype of bluetongue virus (Akita et al., 1994).

In the laboratory, BLU virus can be propagated in different cell cultures such as baby hamster kidney (BHK), Vero, and HmLu-1 (hamster lung) cells, where it produces a clear cytopathic effect (CPE) in 3 to 7 d. No information was found in the literature about the possibility of propagating this virus in canine cells.

In the present study, various experiments were done in hamster lung and canine kidney (MDCK) cell cultures with standard BLU serotypes and the strain isolated from the contaminated vaccine. The purpose of these experiments was to determine whether any differences exist among the prototype BLU strains and the strain isolated from the contaminated vaccine.

MATERIALS AND METHODS

Cell cultures. HmLu-1 cell line, derived from hamster lung, are known for their susceptibility to various arboviruses; these cells were kindly donated by Professor Y. Inaba, Nihon University, Fujisawa, Japan. BHK and Vero cells were from our laboratory's collection. MDCK (American Type Culture Collection, Rockville, MD, CCL 34) is a cell line derived from canine kidney by S. H. Madin and N. B. Darby. These cell lines were generally passaged once a week using a mixture of Trypsin 0.25% + EDTA 0.02% and split at a ratio of 1:3 to 1:5. The cells were grown in a mixture of equal parts of McCoy (5A) and Leibovitz (L15) media supplemented with 5% fetal bovine serum (FBS), 4% 1 *M* HEPES buffer, and 1% of a solution of penicillin-streptomycin (10 000 U and 10 000 μg/ml). The cells formed a complete monolayer usually 24–48 h after being passaged. To infect cell monolayers with virus-containing, or suspected virus-containing material, growth medium on the monolayer was discarded; then a 1/10 dilution of the material was inoculated on the monolayer. Incubation was for 45–60 min at 36° C. After this adsorption, the inoculum was removed, the cultures were washed twice with phosphate buffered saline (PBS), and maintenance medium (1 part McCoy, 4 parts Leibovitz, 1% FBS, 4% HEPES, and 1% antibiotics) was added. The inoculated cultures were reincubated at 33° C and checked daily for CPE. Where indicated, CPE was graded on a scale of 0, 1+ through 4+; 0 = no CPE, 1+ = 25% CPE, 2+ = 50% CPE, 3+ = 75% CPE, 4+ = 100% CPE.

Virus. Bluetongue virus prototype serotypes 2, 10, 11, 13, and 17 (Arthropod Borne Animal Diseases Laboratory, USDA, Denver, CO) were used as standard BLU strains. Stocks of these strains were prepared on HmLu-1 cells, divided in small aliquots, and stored at 4° C.

The BLU isolate from canine vaccine (BLU-vac) was received in a homogenate of dead embryonated chicken eggs (ECE). The ECE had been inocu-

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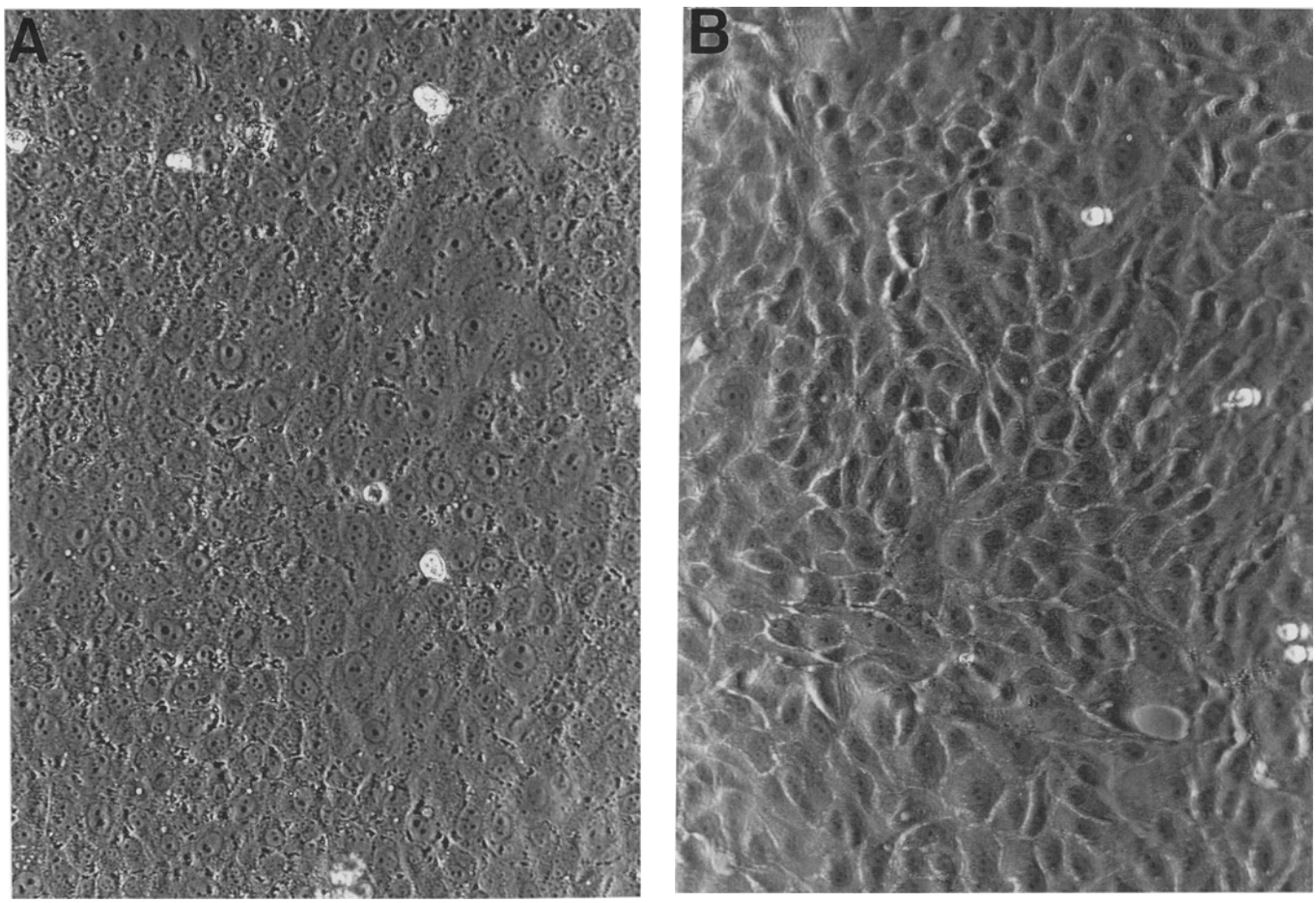

FIG. 1. *A*, Normal MDCK and *B*, HmLu-1 cells (×520).

lated 4–6 d earlier with blood from a sheep injected with the canine vaccine suspected to be contaminated with BLU. The ECE homogenate was inoculated into HmLu-1 cells and caused 100% CPE in 6 d. After two serial passages in HmLu-1 cells, the virus was identified as BLU serotype 11 using a virus neutralization test in cell culture (Akita et al., 1994). A stock of this BLU-vac was prepared in HmLu-1 cells, divided in 1 ml aliquots, and stored at 4° C.

Virus titration. The titer of BLU virus was determined by inoculation of triplicate serial dilutions of virus on HmLu-1 cells in 96-well microtiter plates. CPE was assessed at 6 d after virus inoculation. Virus titer was calculated by the method of Karber (Hawkes, 1979).

The polymerase chain reaction (PCR). In order to detect and identify the BLU virus in MDCK cultures in various experiments, a BLU-specific PCR assay was performed. The procedure for viral RNA extraction and BLU PCR were described previously (Akita et al., 1992).

Experiment 1: Propagation of BLU-vac and BLU 11 prototype in MDCK and HmLu-1 cells. Two cell culture flasks, one with HmLu-1 and the second with MDCK, were inoculated with the BLU-vac isolate. A third flask with MDCK was inoculated with the standard strain of BLU 11. The cultures were checked daily for 8 d for CPE. Regardless of the presence or absence of CPE, samples of the supernatants were collected and reinoculated on HmLu-1 cells to check for proliferation of the inoculated viruses.

Experiment 2: Serial passages of BLU-vac in MDCK cells. Supernatant from the MDCK culture inoculated with BLU-vac isolate in experiment 1 was reinoculated onto MDCK cells. Four serial passages were done in the same manner, each time by inoculating supernatant from the previous passage on MDCK into fresh MDCK cultures. All MDCK cultures were monitored for CPE. In a similar manner, the MDCK supernatant from experiment 1, and aliquots of the supernatants from each serial passage on MDCK cells, were inoculated onto susceptible HmLu-1 cells and monitored for CPE.

Experiment 3: Persistence of BLU-vac in MDCK cells. The MDCK cells from the fourth serial passage of BLU-vac on such cultures from the second experiment were dispersed with trypsin-versene. A portion of the cells were seeded, without any virus inoculation, onto new MDCK cell cultures. At 10–14 d intervals, the same method was used to prepare a subsequent culture. At various intervals after seeding the flask, samples of supernatant were collected to test for the presence of BLU-vac by inoculating them onto susceptible HmLu-1 cultures as described above. An aliquot of MDCK cells from the fourth serial passage, and negative control MDCK cells, were tested for the presence of BLU by BLU-specific PCR as described above. Six serial passages were performed over 2½ mo.

Experiment 4: Continuous replication of BLU-vac in MDCK cultures. A flask from the fourth passage and one from the fifth passage used in the third experiment were not further passaged, but their medium was changed every 3–4 d. Samples from the spent medium, and 1 h after addition of fresh medium, were tested for the presence of BLU-vac by inoculation onto susceptible HmLu-1 cultures as described above.

Experiment 5: Comparison of BLU-vac and five BLU prototype strains. The BLU-vac isolate and the five prototype BLU serotypes isolated in the United States were inoculated in parallel into MDCK and HmLu-1 cells. As in previous experiments, supernatants from the MDCK cultures in which no CPE was detected were reinoculated onto susceptible HmLu-1 cells as described above, in order to test for the presence of BLU virus.

Experiment 6: Cultivation of BLU strains in mixed cultures of MDCK and HmLu-1 cells. Two different types of mixed cultures were prepared: intermixed MDCK and HmLu-1 cell cultures (MDCK + HmLu-1), and side-by-side MDCK and HmLu-1 cell cultures (MDCK/HmLu-1).

1. MDCK + HmLu-1 cultures: Freshly dispersed cells from HmLu-1 and MDCK cultures were mixed in equal volumes and seeded in cell culture flasks. In 2–4 d, a monolayer of contiguous islands of MDCK and HmLu-1 cells was obtained. The origin of the cells was difficult to recognize in the first 2 d, when only isolated cell islands were present. When a complete monolayer was formed, cell types could be distinguished by differences in morphology: the hamster cells were larger and better delineated than the dog cells (Fig. 1 *A,B*).

2. MDCK/HmLu-1 cultures: To obtain better differentiation between the MDCK and HmLu-1 cells in mixed cultures, a second method was used. MDCK cells were seeded in a cell culture flask, and the flask was placed in the CO_2 incubator at a 45° slant so that the cell suspension covered only half of the flask. After 3–4 h, when most of the cells had adhered to the substrate, the medium was discarded and replaced with a fresh suspension of HmLu-1 cells. The flask was reincubated at an opposite 45° slant, so that the medium covered the surface that was not covered previously by the MDCK cells. After 2 h, this medium was discarded and replaced with fresh growth medium; the flask was then incubated horizontally. In 2–3 d, depending on the number of cells seeded, the flask was covered on one-half with dog cells and on the other half with hamster cells. At the interface of these cells, there were interspersed islands of HmLu-1 and MDCK cells. Although the cells were in contact, no overlap occurred for the first 5–6 d (Fig. 2 *A*).

Both kinds of mixed cultures were incubated with BLU-vac and standard strains of BLU and checked for CPE. Mock infected mixed cultures and MDCK and HmLu-1 cell cultures served as controls.

In further experimentation with MDCK and HmLu-1 mixed cultures, the cells were mixed in various proportions to see if the ratio between them had any influence on the appearance and development of CPE in MDCK cells. Different volumes of MDCK and HmLu-1 cell suspensions (ml MDCK + ml HmLu-1) were mixed in a final volume of 6 ml/25 cm flask: 6 + 0, 5.75 + .25, 5.5 + .5, 5 + 1, 4 + 2, 3 + 3, and 2 + 4. All of the flasks were inoculated at the same time with the standard strain of BLU 11 diluted 1:5 and checked daily for CPE. BLU titer was determined in supernatants collected at 5 d postinoculation (DPI).

Experiment 7: Cultivation of BLU strains in mixed cultures of MDCK and Vero or BHK cells. An experiment was done to determine if the CPE caused by BLU on MDCK cells in the mixed MDCK and HmLu-1 cultures was specific to the HmLu-1 cells or if similar results could be obtained with other BLU susceptible cell lines. Mixed cultures were prepared by seeding together equal volumes of MDCK and Vero, BHK, or HmLu-1 cells. At 3 d, the monolayers that formed were inoculated with the reference BLU type 11 and BLU-vac isolate. The virus-inoculated mixed cultures were monitored for CPE. Mock infected mixed cultures, MDCK cell cultures inoculated with BLU type 11 and BLU-vac, served as controls.

Results

Experiment 1. The HmLu-1 culture inoculated with BLU-vac showed a distinct CPE at 4 d postinoculation (DPI). No CPE was seen in the MDCK cultures inoculated with BLU-vac or the standard BLU 11 at any time postinoculation. Supernatant from each MDCK culture inoculated on HmLu-1 cells produced CPE, indicating that both strains of BLU were able to propagate, or at least survive, in MDCK cells, even in the absence of CPE.

Experiment 2. CPE was not observed in any of the MDCK cultures over four serial passages, but CPE was observed in all HmLu-1 cultures. CPE in HmLu-1 cultures appeared on DPI 4 or 5, indicating that the MDCK cells supported replication of the BLU-vac isolate through at least four passages.

Experiment 3. No CPE was observed in any of the MDCK cultures over six serial passages. BLU could be detected repeatedly through all these passages when samples of medium collected at various intervals were inoculated onto HmLu-1 cells (results not shown). MDCK cells that were not inoculated with BLU were negative by BLU-specific PCR; however, the cells from the fourth passage of the persistently infected MDCK cells were positive by the same test.

Experiment 4. The cells in the two MDCK flasks, from passages number four and five of the third experiment, remained in good condition until the end of the experiment with medium changes only. BLU was detected in all medium samples collected at all medium changes, except for a few samples collected 1 h after fresh medium was placed in the flask.

Experiment 5. All BLU strains (type 2, 10, 11, 13, and 17) produced 4+ CPE on the HmLu-1 cells as early as 5–6 DPI. None of the MDCK cells showed any CPE even after 8–9 DPI, but the presence of BLU in these cultures was verified by inoculating supernatant onto susceptible HmLu-1 cultures. The results are presented in Table 1.

Experiment 6. Flasks with HmLu-1, MDCK, MDCK/HmLu-1, and MDCK + HmLu-1 were inoculated with the U.S. prototype BLU serotypes and the BLU-vac isolate. A clear CPE was observed in all HmLu-1 cultures by 4–6 DPI. In the MDCK/HmLu-1 and MDCK + HmLu-1 cultures, the CPE appeared first in the HmLu-1 field or islands (4–6 DPI). Shortly thereafter (5–8 DPI), the MDCK areas or islands also showed CPE (Table 2 and Fig. 2 *B,C,D,E*). No CPE was recorded in any of the BLU-inoculated MDCK flasks up to 9 d (Fig. 2 *F*). All the mock infected control cultures remained negative for CPE for the duration of the experiment. In the mock infected control MDCK + HmLu-1 cultures, the MDCK cells tended to overgrow the HmLu-1 islands, which became smaller or even disappeared. This experiment was repeated three times with similar results. As in previous experiments, virus from the inoculated MDCK cultures without CPE was detected by reinoculating the supernatant onto susceptible HmLu-1 cultures. In the cultures in which various proportions of MDCK and HmLu-1 cells were mixed, no CPE appeared in those cultures with only MDCK cells. Even in the flasks where the ratio between the MDCK and HmLu-1 cells was 23:1, CPE was observed (CPE 2+ at 8 DPI) (Table 3). BLU titer from these cultures determined at 5 DPI demonstrated an increasing trend with increasing CPE.

In order to verify whether the BLU-vac collected from the mixed cultures, which produced CPE in MDCK cells as well as in HmLu-1 cells, could now produce CPE in cultures only with MDCK cells, supernatant from MDCK/HmLu-1 and MDCK + HmLu-1 cultures was reinoculated onto MDCK cultures. No CPE was observed, but again the presence of virus was demonstrated by inoculation of supernatant from the MDCK cultures onto HmLu-1 cultures (results not shown).

Experiment 7. In all three types of cultures (MDCK + HmLu-1, MDCK + BHK, and MDCK + Vero), the monolayer was formed by contiguous islands of cells that could be distinguished on the basis of their morphology. After inoculation, CPE appeared in HmLu-1, Vero, and BHK cultures and also in all three types of mixed cultures. No CPE was observed in the inoculated cultures where only MDCK cells were seeded. All the mock infected cultures remained normal for 8 d (Table 4). A slight delay in the onset of CPE was observed in some of the cultures inoculated with BLU 11 but, at 7 DPI, CPE was complete (Table 4).

Discussion

The first two experiments demonstrated that the virus isolated from the dog vaccine was able to propagate in canine kidney cell cultures.

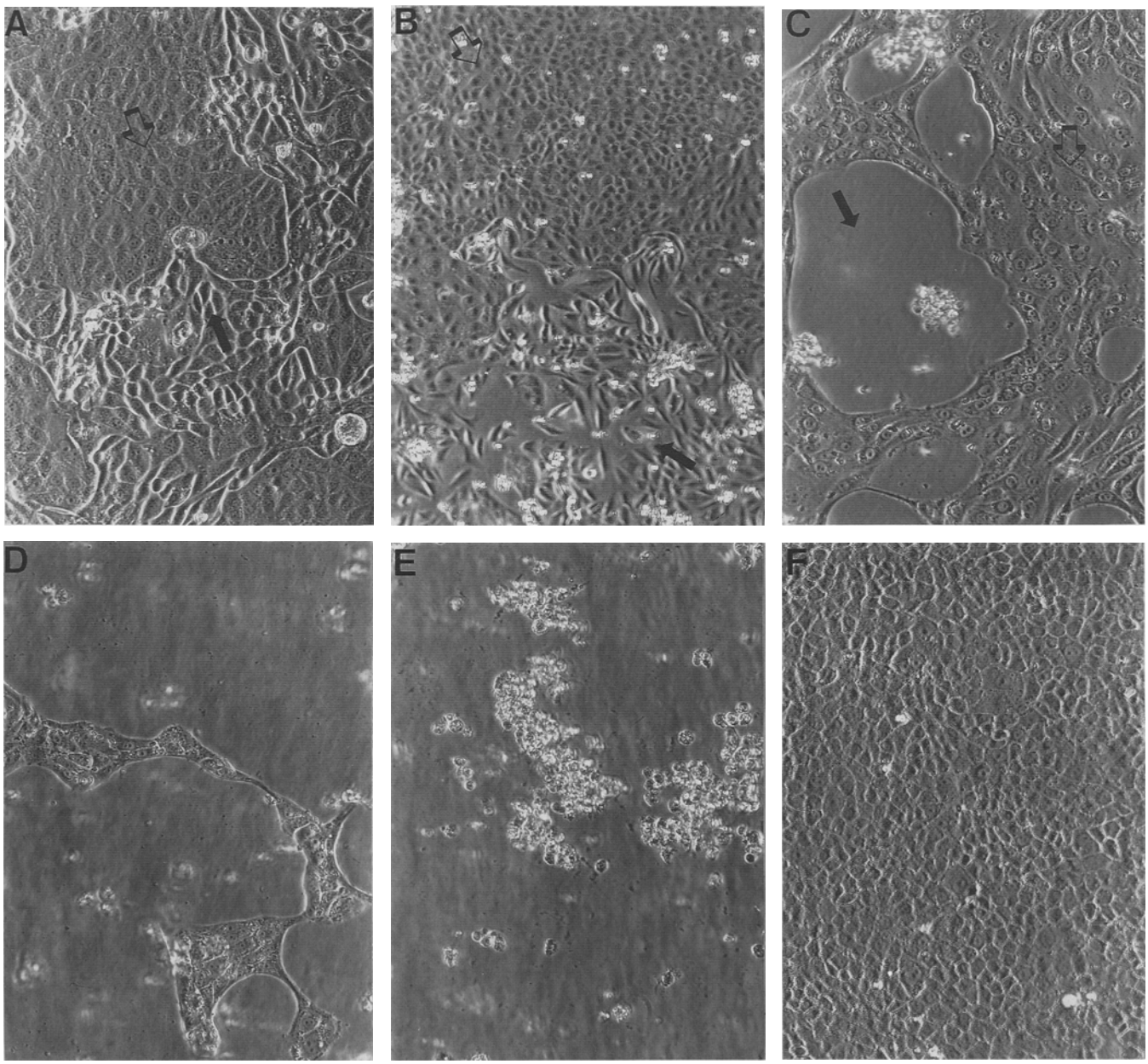

FIG. 2. *A*, Uninoculated MDCK/HmLu-1 mixed culture. MDCK area (*open arrow*) and HmLu-1 area (*closed arrow*). *B*, Mixed MDCK + HmLu-1 culture 3 DPI: Beginning of CPE in the HmLu-1 area (*closed arrow*) but still normal MDCK area (*open arrow*). *C*, Mixed MDCK + HmLu-1 culture 5 DPI: Complete CPE of HmLu-1 islands (*closed arrow*), MDCK cells not yet affected (*open arrow*), *D*, Partial CPE of MDCK areas in mixed culture, 6 DPI; HmLu-1 cells no longer visible. *E*, Complete CPE of both MDCK and HmLu-1 cells, 8 DPI. *F*, MDCK culture at 8 DPI inoculated with the same virus as culture in Fig. 2 *E*; no CPE. All photos ×520.

The fact that virus could be detected after four serial passages eliminated the possibility of persistence of virus from the initial inoculum. Moreover, the third and fourth experiments showed that even in the absence of CPE or any morphological modification of the MDCK cells, BLU-vac caused a persistent infection in MDCK cells, and that these cells continuously produced virus. The fifth experiment showed that not only was the virus isolated from the dog vaccine (BLU-vac), but also all five prototype serotypes of BLU were able to propagate in canine kidney cells without causing CPE.

Of special interest are the results of BLU-vac cultivation in mixed cultures. It was interesting that CPE was also observed in the MDCK cells in such cultures, and this prompted the repetition of such experiments in various forms. Even a relatively small ratio of HmLu-1 cells (Table 3) was able to catalyze the appearance of CPE in MDCK cells. It appeared that the CPE in MDCK cells was less and appeared later in the cultures where fewer HmLu-1 cells were seeded (experiment 6). The reason for this observation is not known. Further, when BLU-vac was collected from mixed cultures in which the MDCK cells

TABLE 1

CYTOPATHIC EFFECT (CPE) OF VARIOUS STRAINS OF BLU GROWN IN MDCK AND HmLu-1 CELL CULTURES (EXPT 5)

Cell Culture and Inoculum	CPE (DPI)[a]	CPE (DPI) after Further Passage on HmLu-1[b]
HmLu-1 + BLU-vac	4+ (7 DPI)	ND[c]
MDCK + BLU-vac	0 (9 DPI)	4+ (6 DPI)
HmLu-1 + BLU 2	3+ (7 DPI)	ND
MDCK + BLU 2	0 (8 DPI)	3+ (6 DPI)
HmLu-1 + BLU 10	4+ (6 DPI)	ND
MDCK + BLU 10	0 (8 DPI)	4+ (5 DPI)
HmLu-1 + BLU 11	4+ (6 DPI)	ND
MDCK + BLU 11	0 (8 DPI)	4+ (5 DPI)
HmLu-1 + BLU 13	4+ (6 DPI)	ND
MDCK + BLU 13	0 (9 DPI)	4+ (5 DPI)
HmLu-1 + BLU 17	4+ (6 DPI)	ND
MDCK + BLU 17	0 (8 DPI)	4+ (6 DPI)

[a]DPI = Days postinoculation.
[b]From the MDCK cultures, in which no CPE was observed, supernatant was inoculated onto fresh HmLu-1 cultures to test for virus proliferation.
[c]ND = Not done; 0, 1+ through 4+ = degree of CPE.

TABLE 2

CYTOPATHIC EFFECT (CPE) PRODUCED BY VIRUS STRAINS OF BLU IN HmLu-1 AND MDCK CULTURES AND MIXED CULTURES (EXPT 6)

Cell Cultures and Inoculum	CPE (DPI)[a]	CPE (DPI) after Further Passage on HmLu-1[b]
HmLu-1 + BLU-vac[c]	4+ (6 DPI)	ND[d]
MDCK + BLU-vac	0 (8 DPI)	4+ (6 DPI)
MDCK/HmLu-1[e] + BLU-vac	3/4+ (7 DPI)	ND
MDCK+HmLu-1[g] + BLU-vac	3+ (6 DPI)	ND
HmLu-1 + BLU 10	4+ (5 DPI)	ND
MDCK + BLU 10	0 (8 DPI)	4+ (6 DPI)
MDCK/HmLu-1 + BLU 10	2/4 (7 DPI)	ND
HmLu-1 + BLU 11	4+ (4 DPI)	ND
MDCK + BLU 11	0 (8 DPI)	4+ (6 DPI)
MDCK/HmLu-1 + BLU 11	3/4+ (7 DPI)	ND
MDCK+HmLu-1 + BLU 11	4+ (6 DPI)	ND
HmLu-1 + BLU 13	4+ (4 DPI)	ND
MDCK + BLU 13	0 (8 DPI)	4+ (6 DPI)
MDCK/HmLu-1 + BLU 13	3/4+ (7 DPI)	ND
MDCK+HmLu-1 + BLU 13	4+ (6 DPI)	ND
HmLu-1 + BLU 17	4+ (5 DPI)	ND
MDCK + BLU 17	0 (8 DPI)	4+ (8 DPI)
MDCK/HmLu-1 + BLU 17	2/4 (7 DPI)	ND
HmLu-1 control	0 (8 DPI)	ND
MDCK control	0 (8 DPI)	ND
MDCK/HmLu-1 control	0 (8 DPI)	ND
MDCK+HmLu-1 control	0 (8 DPI)	ND

[a]DPI = Days postinoculation.
[b]From the MDCK cultures, in which no CPE was observed, supernatant was inoculated onto fresh HmLu-1 cultures to test for virus proliferation.
[c]Viruses were inoculated 1:10.
[d]ND = Not done; 0, 1+ through 4+ = degree of CPE.
[e]MDCK/HmLu-1 mixed cell cultures where half of the flask is covered with MDCK cells and the other half with HmLu-1 cells.
[f]CPE 3+ (75% cell destruction) in MDCK area and 4+ (100% cell destruction on HmLu-1 area).
[g]MDCK+HmLu-1 = mixed culture, in which islands of MDCK and HmLu-1 cells are intermixed.

TABLE 3

GROWTH OF BLU SEROTYPE 11 IN MIXED CULTURES OF MDCK AND HmLu-1 CELLS IN VARIOUS PROPORTIONS (EXPT 6)

ml of MDCK or HmLu-1 seeded			
MDCK	HmLu-1	CPE (DPI)[a]	Titer[b]
6	0	0 (8 DPI)	2.2
5.75	.25	2+ (8 DPI)	3.8
5.5	.5	3+ (8 DPI)	4.2
5	1	4+ (5 DPI)	5.8
4	2	4+ (5 DPI)	5.2
3	3	4+ (5 DPI)	6.2
2	4	4+ (5 DPI)	6.8

[a]Cytopathic effect at day postinoculation.
[b]Titer of BLU in supernatant collected at 5 DPI.

TABLE 4

CYTOPATHIC EFFECT (CPE) OF BLU 11 AND BLU-vac GROWN ON MDCK, HmLu-1, BHK, VERO, AND MIXED CULTURES (EXPT 7)

Cell Culture and Inoculum	CPE at DPI	
	4	7
MDCK + BLU 11	0[a]	0
MDCK + BLU-vac	0	0
HmLu-1 + BLU 11	4+	
HmLu-1 + BLU-vac	4+	
MDCK+ HmLu-1 + BLU 11	4+	
MDCK+HmLu-1 + BLU 11-vac	4+	
BHK + BLU 11	4+	
BHK + BLU-vac	4+	
MDCK+BHK + BLU 11	3+	4+
MDCK+BHK + BLU-vac	3+	4+
Vero + BLU 11	2–3+	4+
Vero + BLU-vac	4+	
MDCK+Vero + BLU 11	2+	4+
MDCK+Vero + BLU-vac	4+	

[a]CPE at 4 d postinoculation.

also showed CPE, it was not able to produce CPE when reinoculated on cultures of MDCK cells alone. This finding argues against an interpretation that soluble toxins from damaged HmLu-1 cells were responsible for the observed CPE in the MDCK cells in mixed cultures. It is possible that more passages in mixed cultures may allow better adaptation of BLU-vac to MDCK cells, so that the virus could cause CPE on MDCK cells.

The results of the seventh experiment showed that the MDCK cells undergo CPE not only when cocultivated with HmLu-1 cells but also when cocultivated with other BLU susceptible cells such as BHK and Vero cells.

The observations reported in this study bring to attention a very important practical aspect concerning the safety of live viral vaccines prepared in cell cultures. Other viruses are known to induce persistent but silent viral infections in cell cultures (SV40 in monkey cells, Avian leukosis virus in chicken embryo fibroblasts). The hazards posed by such silent contaminating viruses is substantial and is of special interest when vaccines are prepared in cell cultures.

Safety controls have been designed in order to detect such contaminants (Johnson et al., 1977; Garrett and Swindells, 1977); most of these tests were developed following reports of cases in which contamination of the vaccine caused accidents. In our case, the contaminant, a virus reported to affect only ruminants, caused lethal disease in dogs.

The method for detecting an occult noncytopathogenic virus in cell cultures by cocultivation with a sentinel cell culture was already described for detecting defective viruses such as in the NP test for avian leukosis (Payne and Purchase, 1991) or other oncoviruses (Rangan et al., 1972; Horodniceanu et al., 1977). This method was also useful for other viruses like BLU in C6/36 mosquito cells. BLU virus could be detected in such cultures by using mixed C6/36 and HmLu-1 cultures (unpublished data).

The use of mixed MDCK and HmLu-1 cultures to reveal the occult BLU viral infection in the present study suggests this method as a supplementary safety test for testing the cell substrate used in vaccine production. By designing a battery of test cell cultures known for their wide susceptibility to various viruses, such occult virus infections could more easily be detected.

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References

Akita, G. Y.; Chinsangaram, J.; Osburn, B. I., et al. Detection of bluetongue virus serogroup by polymerase chain reaction. J. Vet. Diag. Invest. 4:400–405; 1992.

Akita, G. Y.; Ianconescu, M.; MacLachlan, N. J., et al. Bluetongue disease in dogs associated with contaminated vaccine. Vet. Rec. 134:283–284; 1994.

Garrett, A. J.; Swindells, D. Reverse transcripyase and thymidine kinase as markers for tumorigenicity and viral contamination of cells. In: Joint WHO/IABS symposium on standardization of cell substrates for the production of virus vaccines. Dev. Biol. Stand. 37:205–210; 1977.

Hawkes, R. A. General principles underlying laboratory diagnosis of viral infections. In: Lennette, E. H.; Schmidt, N. J., eds. Diagnostic procedures for viral, rickettsial and chlamydial infections. 5th ed. Washington, DC: Am. Public Health Assn.; 1979:3–48.

Horodniceanu, F.; Sinoussi, F.; Chermann, J. C., et al. Replication of primate oncornavirus SSV-1 on MRC-5 human diploid cells. Dev. Biol. Stand. 37:201–204; 1977.

Johnson, R. W.; Benton, C. V.; Perry, A., et al. Contamination monitoring and control in a large scale tissue culture virus production laboratory. In: Acton, R. T.; Lynn, J. D., eds. Cell culture and its application. New York: Academic Press; 1977:571–587.

Knudson, D. L.; Monath, T. P. Orbiviruses. In: Field, B. N.; Knipe, D. M., eds. Virology. Vol. 2. New York: Raven Press; 1990:1405–1433.

MacLachlan, N. J.; Barratt-Boyes, S. M.; Brewer, A. W., et al. Bluetongue virus infection in cattle. In: Walton, T. E.; Osburn, B. I., eds. Bluetongue, African horse sickness, and related orbiviruses. Boca Raton, FL: CRC Press; 1991:725–736.

Payne, L. N.; Purchase, H. G. Leukosis/sarcoma group. In: Calnek, B. W., ed. Diseases of poultry. 9th ed. Ames, IA: Iowa State University Press; 1991:386–439.

Rangan, R. S.; Wong, M. C.; Ueberhorst, P. J., et al. Mixed culture cytopathogenicity induced by virus preparations derived from cultures infected by simian sarcoma virus. J. Natl. Cancer Inst. 49:571–577; 1972.

Roy, P. Towards the control of emerging bluetongue disease. London: Oxford Virology Publications; 1991:1.