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Highly Sensitive Fetal Goat Tongue Cell Line for Detection and Isolation of Foot-and-Mouth Disease Virus[▽]

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A fetal goat cell line (ZZ-R 127) supplied by the Collection of Cell Lines in Veterinary Medicine of the Friedrich Loeffler Institute was examined for susceptibility to infection by foot-and-mouth disease (FMD) virus (FMDV) and by two other viruses causing clinically indistinguishable vesicular conditions, namely, the viruses of swine vesicular disease and vesicular stomatitis. Primary bovine thyroid (BTY) cells are generally the most sensitive cell culture system for FMDV detection but are problematic to produce, particularly for laboratories that infrequently perform FMD diagnostic tests and for those in countries where FMD is endemic that face problems in sourcing thyroid glands from FMD-negative calves. Strains representing all seven serotypes of FMDV could be isolated in ZZ-R 127 cells with a sensitivity that was considerably higher than that of established cell lines and within 0.5 log of that for BTY cells. The ZZ-R 127 cell line was found to be a sensitive, rapid, and convenient tool for the isolation of FMDV and a useful alternative to BTY cells for FMD diagnosis.

Foot-and-mouth disease (FMD) is a highly contagious infectious disease of cloven-hoofed animals. Typical clinical signs are vesicles and erosions of cutaneous mucosae and hairless parts of the skin, affecting mainly the mouth and the hoofs. While FMD was effectively eradicated from Europe by 1990, the disease retains its status of having the highest economic importance due to the catastrophic effect of an outbreak. FMD is endemic in many countries in Asia, Africa, South America, and the Middle East. There are seven different serotypes, and multiple variants of the virus circulate worldwide, with new variants arising continually. Current vaccines are based on inactivated whole-virus preparations of a particular strain, and the immunity they induce only protects against a limited range of field strains. Although for diagnostic purposes, virus isolation may often be replaced by real-time PCR, it is still a crucial first step for the isolation and characterization of new isolates and the selection of a suitable vaccine. Furthermore, for some countries that do not have established reverse transcription (RT)-PCR in their laboratories, virus isolation is still the most sensitive assay for FMDV.

While infective FMD virus (FMDV) is usually present in high concentrations in fresh vesicular material, amounts found in older lesions, sera, nasal swabs, saliva, and oropharyngeal (probang) samples are much lower, necessitating highly sensitive assay systems for their detection. Currently, the most sensitive cell culture system for FMDV isolation is the primary bovine thyroid (BTY) cell (3, 11, 27). However, primary BTY cells cannot be passaged or easily frozen without impairing their sensitivity (unpublished results). Ensuring that there is always a fresh and suitable batch of primary BTY cells available for diagnostic work is quite laborious and expensive (9). Therefore, most diagnostic laboratories use other cells which are less susceptible to FMDV infection but more convenient to handle, such as other primary cells of bovine, ovine, or porcine origin or permanent cell lines such as BHK-21 (4, 19) or IB-RS-2 (5, 6) and sometimes PK-15 cells or SK-6 cells (17).

A study was performed to evaluate a novel fetal goat tongue cell line (ZZ-R 127) from the Friedrich-Loeffler-Institute (FLI) Collection of Cell Lines in Veterinary Medicine (CCLV) as an FMD diagnostic tool. Experiments were conducted both at the FLI, Insel Riems, Germany, and at the Institute for Animal Health (IAH), Pirbright Laboratory, United Kingdom, and the results are presented here.

MATERIALS AND METHODS

Cells. In Germany, cells were obtained from the Institute of Infectology of the FLI, which maintains a collection of cell lines for veterinary medicine. Each cell line in this collection is identifiable by a distinct CCLV-RIE number. In this study, we compared IB-RS-2 cells (adult pig kidney, CCLV-RIE 103) and BHK subline BHK-21/CT cells (baby hamster kidney, CCLV-RIE 164) with a novel fetal goat tongue cell line (ZZ-R 127, CCLV-RIE 127). The history of the ZZ-R 127 cell line is as follows. Very small pieces from the mucosa of the tip of a goat tongue were used as explants to initiate a cell culture. An outgrowth of polymorphic cells but mostly epithelium-like cell types was observed. After a crisis between the 25th and 30th subcultures, there was an alteration to permanence and cells could be multiplied continuously while maintaining their polymorphic epithelium-like morphology.

The species derivation of all of the CCLV cell lines used in this study was confirmed by sequencing a part of the cytochrome *b* gene, which is contained in the mitochondrial DNA (28).

ZZ-R 127 fetal goat tongue cells were passaged with Iscove's Dulbecco modified Eagle medium (DMEM) and Ham's F12 medium at 1:1 supplemented with 10% fetal calf serum (FCS), pH 7.2 to 7.4, at a split ratio of 1:2. Cell growth took 1 week of incubation at 37°C in 4 to 6% CO_2 to develop a confluent monolayer. After a medium change, the cell monolayer was stable at 37°C for at least 21 and up to 28 days. The seeding density was approximately 5.2×10^4 cells/cm^2.

IB-RS-2 cells were passaged with a 1:1 mixture of MEM (minimal Eagle medium) in Hanks' balanced salt solution (BSS) and MEM in Earle's BSS supplemented with 1.25 g/liter $NaHCO_3$, nonessential amino acids, 120 mg/liter Na-pyruvate, and 10% FCS, pH 7.2 to 7.4. For incubation in CO_2, DMEM is

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TABLE 1. Titration of cell culture-grown FMDV in routinely produced BHK and IB-RS-2 cells in comparison with ZZ-R 127 fetal goat cells at the FLI

FMDV serotype and cell type	Titer (log_{10} $TCID_{50}$/ml) at:		Difference from titer in ZZ-R 127 cells
	24 h	48 h	
O_1 Manisa			
ZZ-R 127	5.0	5.9	
BHK-21	NA[a]	4.2	−1.7
IB-RS-2	NA	4.8	−1.1
A Iran 97			
ZZ-R 127	5.5	8.2	
BHK-21	NA	4.8	−3.4
IB-RS-2	NA	5.9	−2.3
A_{22} Iraq			
ZZ-R 127	5.9	7.2	
BHK-21	NA	4.4	−2.8
IB-RS-2	NA	5.1	−2.1
C_1 Noville			
ZZ-R 127	4.9	7.4	
BHK-21	NA	6.5	−0.9
IB-RS-2	NA	6.5	−0.9
Asia 1 Shamir			
ZZ-R 127	7.9	8.6	
BHK-21	NA	6.7	−1.9
IB-RS-2	5.2	6.7	−1.9
SAT 1 Zimbabwe			
ZZ-R 127	6.1	7.0	
BHK-21	NA	4.2	−2.8
IB-RS-2	NA	4.2	−2.8
SAT 2 Zimbabwe			
ZZ-R 127	5.5	7.0	
BHK-21	NA	4.2	−2.8
IB-RS-2	NA	4.2	−2.8
SAT 3 Zimbabwe			
ZZ-R 127	7.3	7.9	
BHK-21	NA	NA	NA
IB-RS-2	NA	6.0	−1.9

[a] NA, not applicable (no CPE was evident in this system at this stage).

suitable at a split ratio of 1:4 to 1:6. Cell growth took incubation for 56 to 72 h at 37°C in 4 to 6% CO_2 to form a continuous monolayer. After a medium change, the cell monolayer was stable at room temperature for at least 14 days.

BHK-21/CT cells were passaged with a 1:1 mixture of MEM in Hanks' BSS and MEM (Eagle) in Earle's BSS supplemented with 1.25 g/liter $NaHCO_3$, nonessential amino acids, 120 mg/liter Na-pyruvate, and 10% FCS, pH 7.2 to 7.4. For incubation in 5% CO_2, DMEM is suitable at a split ratio of 1:6 to 1:10. Monolayers took 48 to 56 h at 37°C in 4 to 6% CO_2 to complete, showing a regular picture with slight orientation. The stability of the monolayer at 37°C was limited, after a medium change and placement at room temperature, to approximately 10 days.

At the IAH, BTY and IB-RS-2 cells (two subclones termed A and B) were grown as described previously (9, 11).

Viruses. Isolates of all seven serotypes of FMDV were taken either from the German National Reference Laboratory collection or from the collection of the World Reference Laboratory for FMD.

At the FLI, cell culture supernatants of O_1 Manisa, A Iran 97, A_{22} Iraq, C_1 Noville, Asia 1 Shamir, SAT 1 Zimbabwe, SAT 2 Zimbabwe, and SAT 3 Zimbabwe (Tables 1 and 2) and bovine epithelia of A_{87} Castellanos, Asia 1 Shamir, A_{22} Iraq, A_{24} Cruzeiro, C_1 Noville, O_1 Manisa, and A Iran 99 (Table 3) were titrated in BHK-21, IB-RS-2, and ZZ-R 127 cells. The susceptibility of ZZ-R 127 cells to porcine epithelia of O_1 Manisa, Asia 1 Shamir, A_{22} Iraq, and A Iran 97 and a further 25 FMDV cell culture supernatants of various strains (A Turkey 1/2006, A Turkey 10/96, A Argentina 5/2001, A Jordan 3/2006, A Eritrea 2/98, A Malaysia 10/97, A Argentina 1/2001, A_5 Modena, A Egypt 1/2006, O Greece 96, O Israel 2/2007, O Algeria 1/99, A Taiwan 3/97, O Taiwan 1/99, O South Korea 1/2000, O_1 BFS 1860, O Albania 1/96, O Pakistan 2/97, O Iran 16/97, O Bulgaria 1/96, O Turkey 5/2005, O United Kingdom 10/2001, O United Kingdom 34/2001, O Japan 2000, and C Oberbayern) was investigated without titration.

In addition, ZZ-R 127 cells were inoculated with swine vesicular disease virus (SVDV) and vesicular stomatitis virus (VSV) of serotypes Indiana and New Jersey.

At the IAH, epithelial suspensions of FMDV serotypes O UAE 2/2003, A IRN 7/97, C_3 Resende, SAT 1 TAN 44/99, SAT 2 ERI 9/98, SAT 3 BEC 1/65, and Asia 1 MAY 14/92, SVDV serotype UKG 155/80, and VSV serotype New Jersey, Montrose, were titrated on BTY, IB-RS 2, and ZZ-R 127 cells (Table 4).

Virus isolation procedures. At the FLI, samples of all seven FMDV serotypes were tested. Virus isolation was performed according to the Office International des Epizooties manual (23). Virus isolation was performed with freshly prepared confluent monolayers. Either 24-well plates or 12.5-cm^2 T flasks were used. After removal of the medium, except for a minimal residual volume, monolayers were incubated with aliquots of FMDV samples (240 μl) for 1 h at 37°C on a swivel plate. Afterwards, fresh medium without FCS was added and the cultures were incubated for 48 h at 37°C in 5% CO_2. Monolayers were observed microscopically for evidence of a cytopathic effect (CPE), a preliminary reading was carried out after 24 h, and a final reading was carried out after 48 h. The test was considered valid if a CPE was observed within 48 h in the FMDV-positive control, which contained approximately 10 $TCID_{50}$ (50% tissue culture infective doses) and no CPE was observed in the negative control. The specificity of a CPE was confirmed by antigen enzyme-linked immunosorbent assay (ELISA) (8) performed according to the Office International des Epizooties manual (23). In a few cases, a real-time RT-PCR procedure was additionally performed (24, 25). In order to determine the sensitivity of the cells to FMDV, fourfold virus dilutions were prepared in tubes and each dilution was transferred to 4 wells of 24-well plates. Titers were calculated by the Spearman-Kärber method (16).

At the IAH, 10% suspensions of vesicular epithelium representing each of the seven serotypes of FMDV plus SVDV and VSV were prepared as previously described (8). The suspensions were clarified by centrifugation and mixed with an equal volume of glycerol (50%, wt/vol), and the resulting stocks were stored at −20°C. The virus isolates that were employed are shown in Table 4.

Comparative titrations of viruses were performed with ZZ-R 127, primary BTY, and IB-RS-2 cells. Tenfold dilution series of each epithelial suspension were made in 0.04 M phosphate buffer, inoculated onto cell monolayers (prewashed with phosphate-buffered saline, overlaid with 2 ml of serum-free Eagle's maintenance medium), grown in plastic tubes (0.2 ml per tube, four tubes per serial 10-fold dilution), and subsequently rolled continuously at 37°C. The cell cultures were examined microscopically for evidence of a CPE daily for up to 4 days postinoculation, and titers of each virus stock were expressed as $TCID_{50}$/ml.

The virus serotype specificity of random cell culture supernatant antigens arising from the virus titrations was confirmed by ELISA (8).

RESULTS

In order to determine whether the new ZZ-R 127 cell line possesses a higher sensitivity than established cell lines, the infectivity titers of cell culture supernatants were determined at the FLI in three different cell lines at various time points following sample inoculation. As shown in Table 1, the titers recorded in ZZ-R 127 cells were consistently higher than those recorded in the other permanent cell lines, the general range of the increase being 0.9 to 4.0 log_{10}.

Table 2 shows the progress of virus replication over time and a range of virus dilutions in a semiquantitative way. With a few exceptions seen at the highest dilutions, virus replication could be detected in ZZ-R 127 cells after 18 to 24 h by recognition of the development of a CPE, whereas consistently almost no CPE could be observed in BHK-21 and IB-RS-2 cells at this stage. After 48 h, there was a CPE in all three cell lines, but still in ZZ-R 127 cells there was a higher number of CPE-positive

TABLE 2. Development of a CPE arising from the titration of cell culture-grown FMDV in routinely produced BHK and IB-RS-2 cells in comparison with ZZ-R 127 fetal goat cells at the FLI

Cell line and FMDV serotype	Presence of CPE at[a]:											
	24 h						48 h					
	A	B	C	D	E	F	A	B	C	D	E	F
ZZ-R 127												
O_1 Manisa	+++	+++	++	+	−	−	+++	+++	+++	+++	+++	+
A Iran97	+++	++	++	++	+	−	+++	+++	++	+++	+++	+++
A_{22} Iraq	+++	+++	+++	+++	++	−	+++	+++	+++	+++	+++	+++
C_1 Noville	++	−	−	−	−	−	+++	+++	+++	+++	+++	+++
Asia 1 Shamir	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
SAT 1 Zimbabwe	+++	+++	+++	−	−	−	+++	+++	+++	+++	+++	−
SAT 2 Zimbabwe	+++	+++	+++	+	−	−	+++	+++	+++	+++	+++	+++
SAT 3 Zimbabwe	+++	+++	+++	++	+	−	+++	+++	+++	+++	+++	+++
BHK-21												
O_1 Manisa	−	−	−	−	−	−	++	+	+	+	−	−
A Iran97	−	−	−	−	−	−	++	+	+	+	−	−
A_{22} Iraq	−	−	−	−	−	−	+++	++	+	+	−	−
C_1 Noville	−	−	−	−	−	−	+++	++	++	+	−	−
Asia 1 Shamir	−	−	−	−	−	−	+++	++	++	+	−	−
SAT 1 Zimbabwe	−	−	−	−	−	−	+	+	+	+	−	−
SAT 2 Zimbabwe	−	−	−	−	−	−	+++	−	−	−	−	−
SAT 3 Zimbabwe	−	−	−	−	−	−	−	−	−	+++	−	−
IB-RS-2												
O_1 Manisa	−	−	−	−	−	−	+++	+++	++	+	−	−
A Iran97	+	−	−	−	−	−	+++	+++	+++	+++	+++	−
A_{22} Iraq	−	−	−	−	−	−	+++	+++	+++	+++	−	−
C_1 Noville	−	−	−	−	−	−	+++	+++	+++	+++	+++	++
Asia 1 Shamir	−	−	−	−	−	−	+++	+++	+++	+++	++	−
SAT 1 Zimbabwe	−	−	−	−	−	−	+++	+++	+++	+++	+++	−
SAT 2 Zimbabwe	−	−	−	−	−	−	++	−	−	−	−	−
SAT 3 Zimbabwe	−	−	−	−	−	−	+++	+++	+++	+++	++	−

[a] A, B, C, D, E, and F represent dilutions in 0.6-log steps. Symbols: −, no CPE detected; +++, CPE affecting >60% of the monolayer; ++, CPE affecting 20 to 60% of the monolayer; +, CPE affecting <20% of the monolayer.

wells, in particular, with the type SAT 2 and SAT 3 FMDV titrations.

In order to confirm further the suitability of ZZ-R 127 cells for the isolation of FMDV and test a broader range of isolates, the cells were inoculated with 25 additional cell supernatants without titration. In all but one cases, the cells were susceptible to infection with the FMDV isolates and a strong CPE was observed within 24 h. The exception was strain A TAW 3/97, which is completely pig specific and does not infect cattle under field conditions. It did not produce a clear CPE in ZZ-R 127 cells but led to the development of a CPE in IB-RS-2 cells.

In order to exclude the possibility that these results are artifacts that only apply to cell culture-adapted FMDV, at the FLI, all three cell lines were inoculated with different fourfold dilutions of bovine vesicular material (i.e., wild-type FMDV) of A_{87} Castellanos, Asia 1 Shamir, A_{22} Iraq, A_{24} Cruzeiro, C_1 Noville, O_1 Manisa, and A Iran 99, and the results are shown in Table 3.

After 24 h, a CPE could be observed for each sample in ZZ-R 127 cells, but not in IB-RS-2 cells, whereas in BHK-21 cells, only Asia 1 Shamir, A_{24} Cruzeiro, and C_1 Noville led to the development of a CPE. Subsequently, a CPE could be observed in all three cell lines at 48 h postinoculation.

Furthermore, susceptibility to pig-passaged virus was checked with vesicular material from pigs (O_1 Manisa, Asia 1 Shamir, A_{22} Iraq, and A Iran 97). All four isolates showed a CPE in ZZ-R 127 cells after 24 h, whereas only the A Iran 97 isolate showed a CPE in IB-RS-2 cells at this time. No CPE developed after inoculation with SVDV UKG and VSV of serotypes Indiana and New Jersey.

At the IAH, epithelial suspensions of FMDV O UAE 2/2003, A IRN 7/97, C_3 Resende, SAT 1 TAN 44/99, SAT 2 ERI 9/98, SAT 3 BEC 1/65, and Asia 1 MAY 14/92 were titrated on ZZ-R 127 cells in comparison to primary BTY cells, as well as IB-RS-2 cells, and the results are shown in Table 4. Strains representing all seven serotypes of FMDV could be isolated on ZZ-R 127 cells with a sensitivity that was higher than that of IB-RS-2 cells. By calculating the mean of the titers in ZZ-R 127 and BTY cells, respectively, the sensitivity of the goat cells was found to be approximately 0.5 log lower than that of the primary BTY cells. No CPE was observed after inoculation of ZZ-R 127 cells with SVDV UKG 155/80, while VSV New Jersey led to the development of a CPE but only at a high virus inoculum concentration.

DISCUSSION

In general, the most sensitive cells for FMDV isolation are primary BTY cells. However, ensuring their constant availability for diagnostic purposes is laborious and expensive, and there are also batch-to-batch variations in their quality and fitness for the purpose. Therefore, most diagnostic laboratories

TABLE 3. Development of a CPE arising from the titration of epithelial suspensions of FMDV in routinely produced BHK and IB-RS-2 cells in comparison with ZZ-R 127 fetal goat cells at the FLI

Cell line and FMDV serotype	Presence of CPE at[a]:									
	24 h					48 h				
	A	B	C	D	E	A	B	C	D	E
ZZ-R 127										
A_{87} Castellanos	+	+	−	−	−	+	+	+		
Asia 1 Shamir	+	+	+	−	−	+	+	+	−	−
A_{22} Iraq	+	+	+	−	−	+	+	+	+	+
A_{24} Cruzeiro	+	+	+	−	−	+	+	+	+	+
C_1 Noville	+	+	+	+	−	+	+	+	+	−
O_1 Manisa	+	+	+	−	−	+	+	+	+	−
A Iran 99	+	+	+	−	−	+	+	+	−	−
BHK-21										
A_{87} Castellanos	−	−	−	−	−	+				
Asia 1 Shamir	+	−	−	−	−	+	+	−	−	−
A_{22} Iraq	−	−	−	−	−	+	+	+	−	−
A_{24} Cruzeiro	+	−	−	−	−	+	+	+	−	−
C_1 Noville	+	+	+	+	−	+	+	+	+	−
O_1 Manisa	−	−	−	−	−	−	−	−	−	−
A Iran 99	−	−	−	−	−	+	+	−	−	−
IB-RS-2										
A_{87} Castellanos	−	−	−	−	−	+				
Asia 1 Shamir	−	−	−	−	−	+	+	−	−	−
A_{22} Iraq	−	−	−	−	−	+	+	−	−	−
A_{24} Cruzeiro	−	−	−	−	−	+	+	+	−	−
C_1 Noville	−	−	−	−	−	+	+	+	+	+
O_1 Manisa	−	−	−	−	−	+	+	+	−	−
A Iran 99	−	−	−	−	−	+	−	−	−	−

[a] A, B, C, D, and E represent dilutions in 0.6-log steps. Symbols: −, no CPE detected; +, CPE detected.

use other cells which are less sensitive but more convenient to handle, such as cells of bovine, ovine, or porcine origin or permanent cell lines such as BHK-21 or IB-RS-2. These protocols do not usually facilitate reliable or overnight amplification and detection of virus.

Since more reliance is now being placed on real-time RT-PCR procedures for FMDV diagnosis and less is being placed on virus isolation, FMD laboratories that might use them are even less likely than in the past to maintain the propagation of primary BTY cells on a regular basis. If primary BTY cells are not then routinely produced, the danger is that the skills for their propagation will become lost or not so easily used. As for a disease as contagious as FMD, one would like to have a sensitive system in reserve for emergency situations and not rely entirely on PCR, it will be important to have a cell line with equivalent or nearly equivalent sensitivity to that of BTY cells that can either be routinely maintained or quickly resuscitated from the freezer to be made available for diagnostic use. Furthermore, the procedures for vaccine strain selection require isolation of the virus causing the outbreak. As time is a crucial factor for emergency vaccination, saving even 1 or 2 days can make a significant difference in the adoption of control measures.

In this study, we have shown that a fetal goat tongue cell line (ZZ-R 127) was found to be highly sensitive to the replication of both wild-type and cell-adapted FMDV strains. The cell line was validated as a tool for FMDV isolation in cooperation with the IAH. Strains representing all seven serotypes of FMDV could be isolated on ZZ-R 127 cells with a sensitivity that was only slightly inferior to that of BTY cells but significantly higher than that of BHK-21 or IB-RS-2 cells. In total, 40 isolates (14 type A, 16 type O, 2 type C, 2 type Asia, 2 type SAT 1, 2 type SAT 2, and 2 type SAT 3) could be grown on ZZ-R 127 cells, and the results suggest that the cell line has suitable susceptibility to a broad range of FMDV strains. FMDV infection was almost always detected by visual means within 18 to 24 h in the first passage, whereas in BHK-21 and IB-RS-2 cells, the detection of small amounts of FMDV often took several days and may have required additional cell culture passages.

It was concluded that the fetal goat tongue cell line is a sensitive, fast, and convenient tool for the isolation of FMDV which almost matches the sensitivity of BTY cells and has significant advantages over established permanent cell lines.

While the experiments described in this report were done with the 76th to the 91st passages (FLI) and the 141st to the 142nd passages (IAH), the fetal goat tongue cell line maintains its growth characteristics and its sensitivity to FMDV at least from the 76th to the 160th passages and its properties are not dependent on the type of vessel or plasticware used (data not shown). It is possible to resuscitate the cells of a high-density working stock in a way that within 1 to 2 days a susceptible monolayer is achieved.

While no replication of SVDV UKG was observed in ZZ-R 127 cells, VSV led to the development of a CPE when the virus was present at a high concentration. As the new goat cell line is not proposed as a diagnostic tool for SVDV or VSV but for FMDV, this is not considered a disadvantage. At the FLI and

TABLE 4. Titration of epithelial suspensions of FMDV, SVDV, and VSV in routinely produced IB-RS-2 and primary BTY cells in comparison with ZZ-R 127 fetal goat tongue cells at the IAH

Cell culture type[a]	Titer (log_{10} $TCID_{50}$/ml)								
	FMDV							SVDV UKG 155/80	VSV New Jersey
	O UAE 2/2003	A IRN 7/97	C3 Resende	SAT 1 TAN 44/99	SAT 2 ERI 9/98	SAT 3 BEC 1/65	Asia 1 MAY 14/92		
IB-RS-2 (A)	5.7	6.2	2.95	6.95	6.45	3.45	6.2	4.45	ND[b]
IB-RS-2 (B)	5.2	6.2	2.45	6.45	5.2	3.95	5.95	5.7	4.2
Goat tongue ZZ-R 127	5.95	6.45	3.7	6.7	6.7	4.95	6.2	≤2.2	2.2
BTY	5.95	6.7	4.45	7.2	7.2	5.7	6.2	ND	1.95

[a] IB-RS-2, permanent pig kidney cell line (subclones A and B).
[b] ND, not detected.

the IAH, all material of porcine origin is tested also on a porcine cell line susceptible to SVDV. The inoculation of any material of porcine origin onto a suitable cell line of porcine origin is also necessary because some highly pig-adapted strains (e.g., TAW 3/97) do not grow well on cells derived from other species, including ZZ-R 127 cells. On the other hand, the FMDV specificity of any CPE observed will always have to be checked by ELISA or PCR.

The reason why the susceptibility of the goat ZZ-R 127 cell line is considerably higher than that of other permanent cell lines is not yet known. An important factor which determines the susceptibility of a cell line to a particular virus is the expression of suitable surface receptors on the cell surface. Integrin molecules are receptors for a number of viruses, including field isolates of FMDV (13, 15, 20, 22). Integrins are heterodimers comprising alpha and beta subunits, each of which can occur in a number of different forms. Six or seven different varieties of integrin are known to bind to the conserved RGD amino acid motif found on the VP1 capsid protein of FMDV. The integrin αvβ6 is considered to be the principal receptor for the binding of wild-type FMDV (10, 14). Beside integrins, heparan sulfate proteoglycans (HSPGs) (12) are used by FMDV for cell attachment and virus replication (21). However, there is no convincing evidence of a role for HSPG in cell entry by field viruses (1, 2, 7, 18, 22, 26). In order to exclude the possibility that the higher susceptibility of the ZZ-R 127 cell line is due to HSPGs and does not apply to field virus, epithelial suspensions containing FMDV were also included in this study. The results confirmed what was found with cell culture supernatants. Comparative receptor studies with the three cell lines have been initiated, and preliminary results indicate that >90% of ZZ-R 127 cells express the αvβ6 receptor (our unpublished fluorescence-activated cell sorter data).

The overall results suggest that the ZZ-R 127 cell line is almost equal in sensitivity to primary BTY cells for FMDV isolation and could be introduced into FMD diagnostic laboratories worldwide to improve local FMDV isolation procedures.

The ZZ-R 127 fetal goat tongue epithelium cell line is available from the CCLV, FLI, Greifswald-Insel Riems, Germany (catalogue no. RIE 127).

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