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Bovine Coronavirus-Induced Cytopathic Expression and Plaque Formation: Host Cell and Virus Strain Determine Trypsin Dependence

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With 4 figures and one table

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Summary

The cytopathic expression (CPE) and plaque formation of different strains of enteropathogenic bovine coronavirus (BCV) were analyzed in a variety of cell types in the presence and absence of trypsin. The cell-adapted BCV-L 9, originally isolated by Mebus et al. (1973), and 5 wild-type strains replicated in the HRT-18 line of human rectal tumor cells, inducing cytopathic changes with cell fusion, which was enhanced by trypsin. Strain L 9 replicated noncytopathically in D 2 bovine fetal spleen (D 2 BFS) cells, depending absolutely on the presence of trypsin for the activation of cell fusion. D 2 BFS cells were non-permissive for all wild-type BCV strains even in the presence of trypsin. Strain L 9 produced plaques in HRT-18 clones, but the ease of plaque formation and plaque morphology were host-cell dependent. Wild-type BCV was also depended on the type of HRT host cell for plaque formation, and a unique plaque morphology with different degrees of trypsin dependence was evident for each strain. The BCV strain and the host cell determined the degree of dependence on trypsin for enhancement of CPE and plaque formation.

Key words: BCV cytopathic expression, plaques, trypsin dependence

Introduction

The spectrum of cytopathic expression of bovine coronaviruses (BCV) ranges from mild to highly cytocidal according to the virus strain and the host cell. Cytocidal infections may induce cell fusion with ensuing cell lysis. Persistent coronavirus infections also occur in some cell lines (Chaloner-Larsson and Johnson-Lussenburg, 1982; Holmes and Behnke, 1981; Lucas et al., 1978). Many strains of coronaviruses do not replicate in cultured cells. Frana et al. (1985) reported host-dependent processing of the structural proteins of mouse hepatitis virus (MHV-A 59). They found that activation of the viral fusion factor was due in part to cleavage of the 180 Kd peplomer to 90 Kd subunits. Trypsin enhanced the cell-fusing activity of this virus (Frana et al., 1985; Sturman et al., 1985; Yoshikura and Tejima, 1981). A trypsin-dependent increase in cell-fusing activity and plaque development of BCV was demonstrated using bovine fetal brain and bovine fetal thyroid cells (Storz et al., 1981).

The objective of this study was to determine the influence of the host cell and virus strain on trypsin dependence of cytopathic expression and plaque development of BCV. Cells which varied in susceptibility to infection and several wild-type BCV isolates, as well as the cell-adapted strain L 9 were assayed.

Material and Methods

Cell Cultures

The human adenocarcinoma cell line HRT-18 (TOMPKINS et al., 1974) and HRT-18 cell clones 3 F 3, D 2, 3 E 3, and 4 B 3, established by limiting dilution, were maintained in Dulbecco's modified minimum essential medium (DMEM) containing penicillin (100 units/ml) and streptomycin (100 µg/ml). The medium was supplemented with 5 % fetal calf serum. D 2 BFS cells were derived from bovine fetal spleen cells. They are a population of cells which survived precrisis and continue to multiply well past the 30th passage. D 2 BFS cells were maintained in Eagle's minimum essential medium (MEM) containing 25 mM Hepes and penicillin (100 units/ml)-streptomycin (100 µg/ml). MEM was supplemented with 10 % fetal calf serum. No serum was used in the presence of virus.

Virus Strains

BCV-L 9 and 5 wild-type strains (isolated from calves in Colorado and Utah) were used. Wild-type isolates were designated Meeker, Miller, Fisher, Calf 50, and LY-138. Strain LY-138 was maintained by calf inoculation since 1965. These isolates were obtained from diarrhea fluids of sick calves and had been passaged 2—3 times in HRT-18 cells. The original field samples were collected before the modified live BCV of Norden Laboratories was applied as a vaccine. The L 9 strain was isolated and adapted to cell cultures by MEBUS and coworkers (1973, 1976).

Propagation of Virus in Different Cells

Monolayers of HRT-18 parent cells, HRT-clones, and bovine fetal spleen (D 2 BFS) cells in 25 cm^2 flasks were infected in duplicate with BCV. BCV-L 9 was inoculated at an MOI of 3×10^{-3} PFU/cell. Wild-type strains were inoculated at MOIs of 1 and 0.1 PFU/cell. Following 1 h of adsorption, the inoculum fluid was removed, and 5 ml of medium was added. One flask from each set received medium, the other set received medium + trypsin (5 µg/ml trypsin was used with HRT-18 cells; 7.5 µg/ml trypsin was used with D 2 BFS cells). The trypsin concentration used was the maximum concentration of the enzyme which did not produce damage to the monolayers. All flasks were incubated at 37 °C and monitored daily for CPE.

Table 1. Cytopathic expression of BCV strains in different cell types

Cells	Trypsin	L 9	Miller	Meeker	Fisher	Calf 50	LY-138
D 2 BFS	A	±	–	–	–	–	–
	P	++ (ef)	–	–	–	–	–
HRT-18	A	+ (lf)	+ (lf)	+ (lf)	+ (lf)	+ (lf)	+
parent	P	++ (ef)	++ (mf)	++ (ef)	++ (mf)	++ (mf)	++ (mf)
Clone	A	+	ND	ND	ND	ND	ND
4 B 3	P	+					
Clone	A	+ (lf)	ND	ND	ND	ND	ND
D 2	P	++ (mf)					
Clone	A	+ (lf)	ND	ND	ND	ND	ND
3 E 3	P	++ (mf)					
Clone	A	+ (mf)	ND	ND	ND	ND	ND
3 F 3	P	++ (ef)					

\+ = degree of cytopathic change
– = no cytopathic change, no BCV hemagglutinin produced
(lf) = light fusion
(mf) = moderate fusion
(ef) = extensive fusion
A = trypsin absent
P = trypsin present
ND = not done

Plaque Formation

Confluent monolayers of HRT-18 cell clones E F 3, D 2, 3 E 3, and 4 B 3 in 6-well polystyrene plates were infected with 10-fold serially diluted virus (0.5 ml/well) in paired sets. The overlay medium consisted of DMEM + 0.6 % agarose (Gibco, electrophoresis grade). Trypsin (Difco, 1 : 250, 2.0 µg/ml) was added to the overlay of one set. After incubation for 3—4 days, cells were stained for 2 h with neutral red, fixed with formalin saline for 10 min, and the overlays were removed for counting and morphological analysis of the plaques.

Results

Cytopathic Expression of L 9 and Wild-type BCV in Different Cells

The cytopathic expression of five wild-type BCV strains and strain L 9 were analyzed in the presence and absence of trypsin using HRT-18 parent cells, four HRT-18 cell clones, and D 2 BFS cells. The results of this investigation are given in Table 1. HRT-18 cells were permissive to all strains of BCV. These cells were initially used for isolation of wild-type strains from diarrhea fluids. Each strain of virus induced cell fusion in HRT-18 cells, and trypsin enhanced this function. Extensive fusion occurred in HRT-18 cells infected with strains L 9 and Meeker in the presence of trypsin, while moderate fusion was a result of infection with strains Miller, Fisher, Calf 50, and LY-138. The cytopathic expression resulting from infection of HRT-18 cells with BCV-L 9 with and without trypsin is demonstrated in Figure 1. Strain L 9 replicated with varied intensity in the four HRT-18

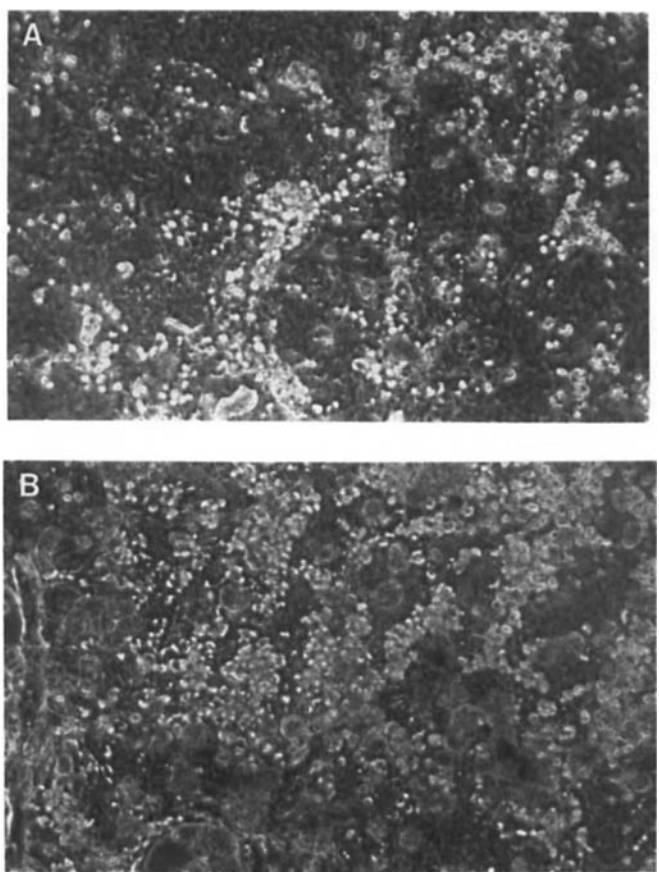

Fig. 1. Phase-contrast photomicrograph of BCV-L 9-infected HRT-18 cells. A. (−) trypsin. B. (+) trypsin. Magnification — 100×

cell clones. Trypsin increased the cytopathic expression in clones 3 F 3, D 2, and 3 E 3, but not in clone 4 B 3. Cell fusion was observed in all clones except clone 4 B 3. D 2 BFS cells were non-permissive to infection by all the field isolates even in the presence of trypsin. Strain L 9 replicated in D 2 BFS cells without cytopathic expression, but cell fusion occurred only in the presence of trypsin (Fig. 2).

Plaque Development under Different Conditions

The formation of plaques by BCV-L 9 was monitored in HRT-18 clones 3 F 3, 4 B 3, 3 E 3, D 2, and 3 E 3 both with and without trypsin to assess any host cell-dependent differences in plaque formation (Fig. 3). The virus produced sharp, distinct plaques of 1—2 mm diameter in clone 3 F 3 within 4 days post infection, but the plaques developed within 2—3 days in the presence of trypsin. The plaque diameter more than tripled, the plaques were less turbid, and they were more sharply defined when trypsin was included in the overlay. Small, turbid plaques of approximately 1—2 mm diameter developed in clone 4 B 3. The plaques were more distinct in the presence of trypsin but the plaques remained turbid, and the diameter was unchanged. Distinct plaques of about 0.5—2 mm diameter were formed by L 9 in clone D 2. Trypsin did not increase the size of these plaques, but the plaques were less turbid and easier to see. Plaques were not evident in the 3 E 3 cell monolayer in the absence of trypsin. Plaques remained indistinct, but they were apparent in wells containing trypsin.

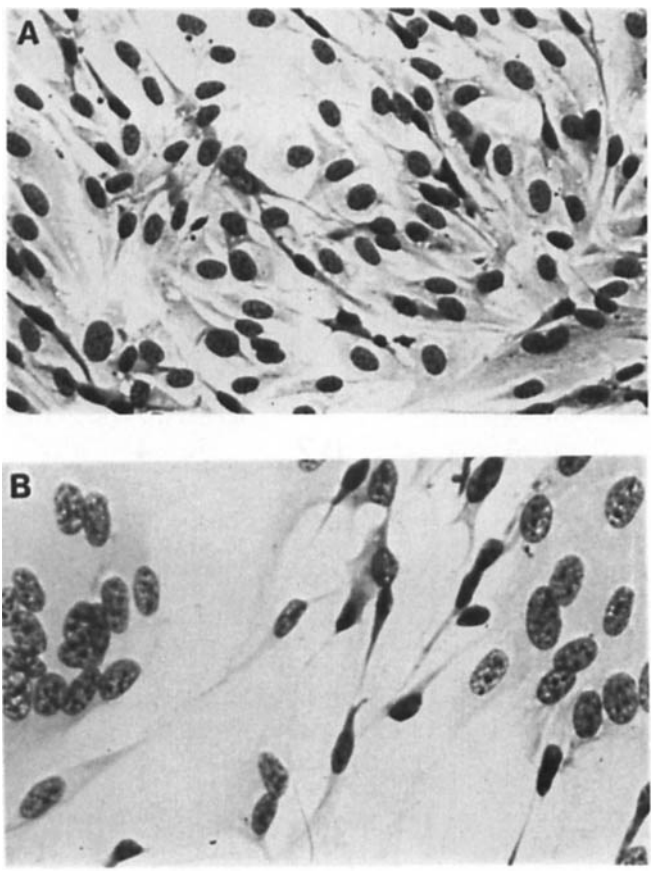

Fig. 2. Photomicrograph of Giemsa-stained cultures of BCV-L 9-infected D 2 BFS cells. A. (–) trypsin. B. (+) trypsin. Magnification — 400 ×

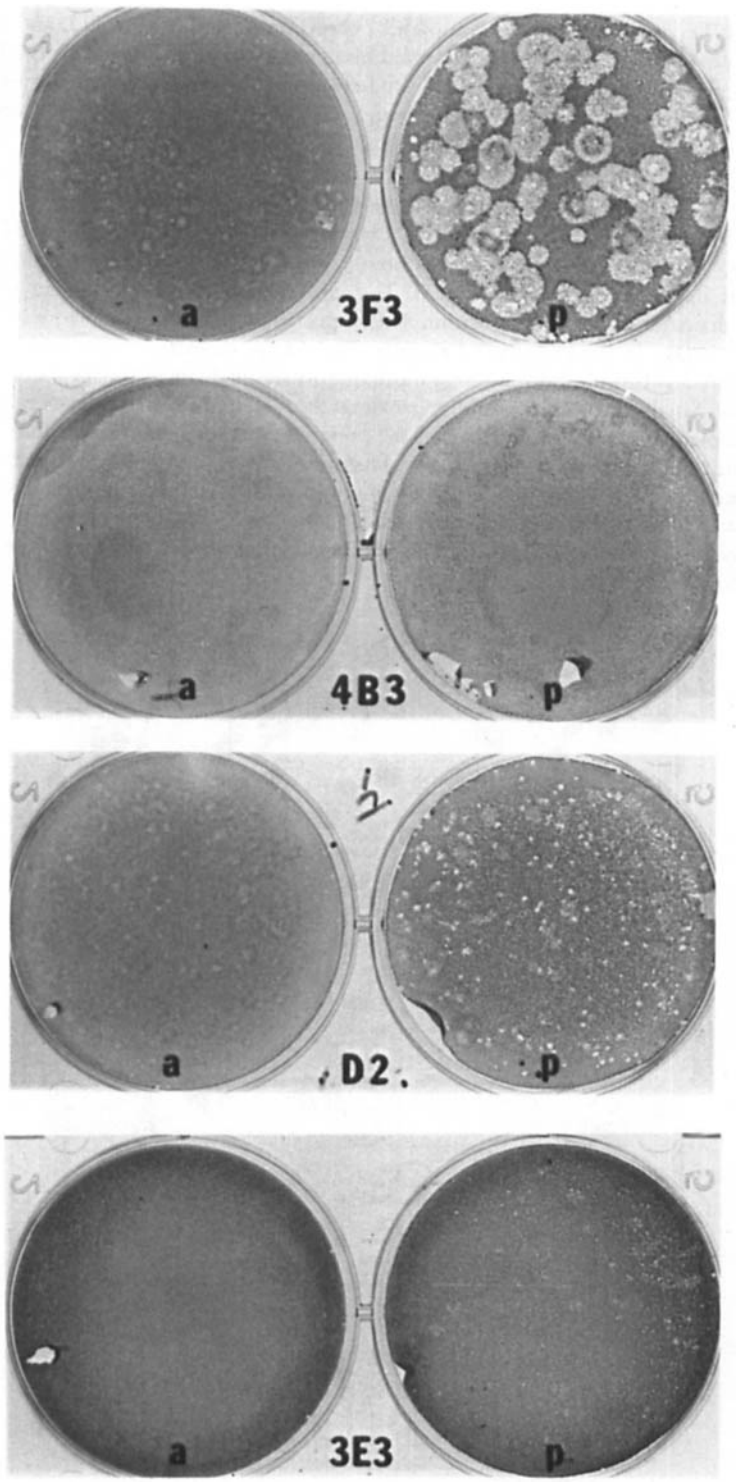

Fig. 3. Plaque development of BCV-L 9 in HRT-18 clones 3 F 3, 4 B 3, D 2, and 3 E 3. a — trypsin absent, p — trypsin present

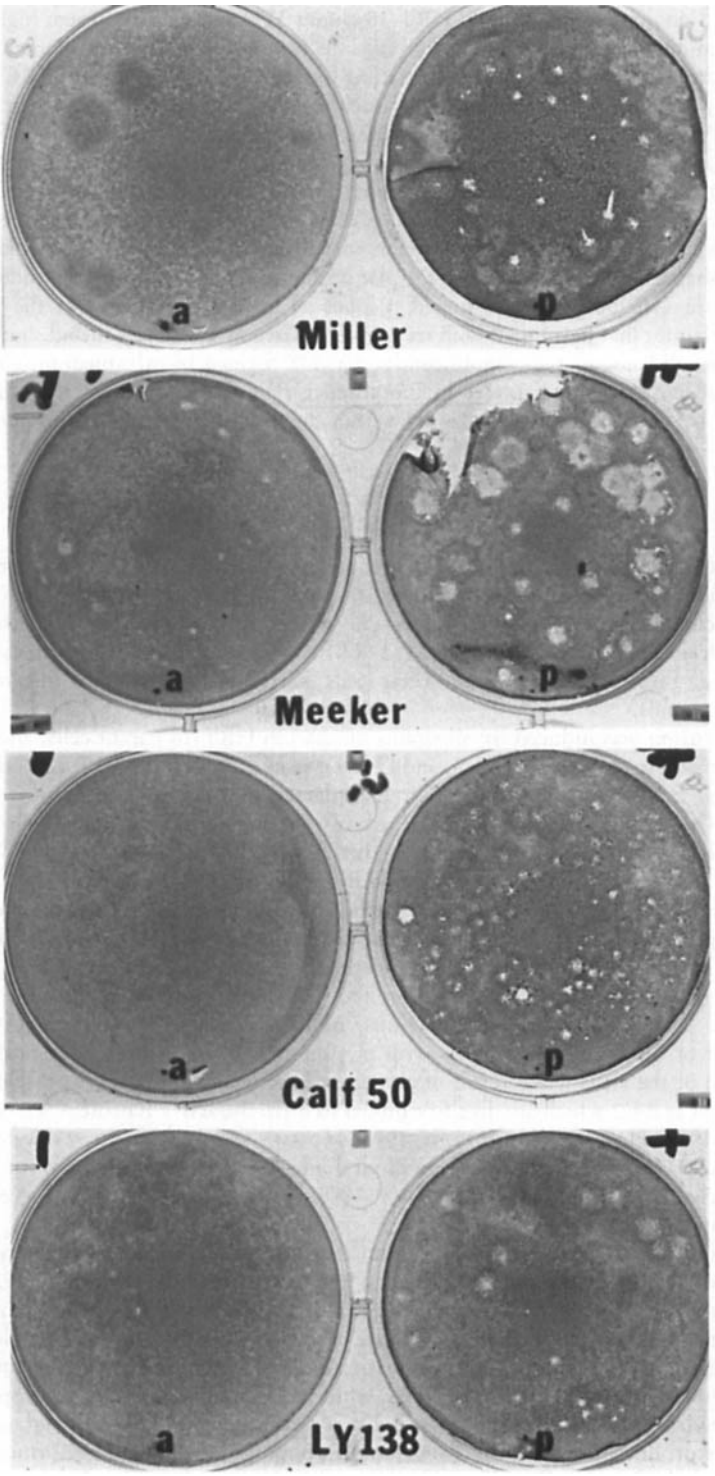

Fig. 4. Plaque development of wild-type BCV strains in clone 3 F 3. a — trypsin absent, p — trypsin present

Based on cytopathic changes, HRT-18 clones 3 F 3 and 4 B 3 represent highly susceptible and minimally susceptible cells to BCV infection, respectively. Consequently, the two clones were used to assess plaque formation by four wild-type strains of BCV. Plaques were not produced by any of the isolates in clone 4 B 3 either in the presence or absence of trypsin. Plaques developed readily, and they were enhanced by trypsin in cells of clone 3 F 3 (Fig. 4). Plaques ranging in size from 2—4 mm were produced by strain Miller. The number of detectable plaques increased with trypsin, but the plaques remained relatively turbid and of similar size to those produced without trypsin. The plaques formed by strain Meeker were 1 mm or 2 mm in diameter with fairly clear centers. These plaques were greatly enhanced by trypsin because the plaque diameters doubled, and the centers became clear. Strain Calf 50 produced turbid plaques of 1—2 mm diameter in the absence of trypsin. Plaques that developed with trypsin in the overlay were less turbid, but the size of the plaques did not increase significantly. LY-138 formed mostly medium-sized, turbid plaques of about 2 mm diameter in the absence of trypsin. Some of the plaques were enhanced by trypsin, but other plaques within the monolayer exhibited no enhancement.

Discussion

Host-dependent differences in the cytopathic expression of BCV were demonstrated in this study. HRT-18 parent cells allowed the replication of all strains of BCV. The four HRT-18 clones were permissive to infection with strain L 9, and the cytopathic changes were different in these cells. In contrast, D 2 BFS cells only allowed the replication of the cell-adapted virus strain, L 9, while these cells were non-permissive to infection by the wild-type strains.

Cell fusion was induced by all strains of virus in HRT-18 parent cells, and strain L 9 induced fusion in clones 3 F 3, 3 E 3, and D 2. Trypsin enhanced the CPE and cell fusion in these cells. Fusion was not evident in L 9-infected clone 4 B 3 either with or without trypsin. These cells may be incapable of fusion due to the composition of the cell membrane (HAYWOOD and BOYER, 1982 a, b; HUANG, 1983; MIYAKE et al., 1978), or they may process the virus structural proteins differently. Cell fusion occurred in L 9-infected D 2 BFS cells only in the presence of trypsin. This finding indicates that these cells lack a specific protease necessary for activating the cell-fusing activity of the virus. This situation is analogous to that found for Sendai virus propagated in L cells. Propagation of chicken embryo-borne Sendai virus in L cells resulted in a single round of replication, and the L cell-borne Sendai virus was noninfectious, nonhemolytic, and did not induce fusion. Treatment of the L cell-borne virus with trypsin activated the three biological activities. Activation of the virus was a result of cleavage of the inactive precursor protein Fo to the subunits F 1 and F 2 which are disulfide linked to form the active F protein (HOMMA, 1971; HOMMA, 1972; HOMMA and OHUCHI, 1973; HOMMA and TAMAGAWA, 1973; SCHEID and CHOPPIN, 1974). Proteolytic activation of viral infectivity was also reported for Newcastle disease virus and orthomyxoviruses (ROTT, 1979; GARTEN et al., 1981; NAGAI et al., 1976; PEEPLES and BRATT, 1984).

Plaque formation was dependent upon the host cell line, the strain of virus, and the presence of trypsin in the overlay. Strain L 9 produced plaques in the four HRT-18 cell clones, but the ease of plaque production and the morphology of the plaques varied between the cell lines. Differences in the rate of replication of the virus in the clones could produce this result. Within a given incubation period, larger plaques would form in cells which allow rapid replication of the virus, while smaller plaques would develop in cells in which the virus replicates slowly. Trypsin dramatically enhanced the size and morphology of plaques produced in clone 3 F 3, but the morphology of the plaques formed in clones D 2, 3 E 3, and 4 B 3 were less affected by trypsin. Enhanced plaque production of BCV-L 9 by trypsin was also demonstrated in bovine fetal brain and thyroid cells (STORZ et al., 1981).

Clone 4 B 3 was nonpermissive to plaque production by the wild-type BCV strains, but plaques developed readily in clone 3 F 3, again demonstrating the host-dependence of BCV replication. Each wild-type strain produced distinct plaque phenotypes that were enhanced by trypsin. Since these virus stocks were not plaque purified, they probably consist of a variety of genotypically distinct viruses.

BCV-L 9 is a highly cell-adapted strain of virus which replicates easily in culture. In contrast, the wild-type strains were isolated from diarrhea fluids and were passaged only 2—3 times in cultures of HRT-18 cells. The wild-type strains differ in cytopathogenicity in culture. WILLIAMS (1983) reported differences in the polypeptide profiles of these viruses. Structural differences may affect the viruses' ability to adsorb to or replicate in a cell type, or affect the ease and extent of processing of viral proteins by the host cell or by trypsin. These factors probably contribute to the differences in cytopathic expression and plaque development observed in this study. One might expect that they also reflect differences in the enterpathogenic potential of BCV strains for calves.

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Zusammenfassung

Zytopathische Expression und Plaquebildung des bovinen Coronavirus: Wirtszelle und Virusstamm bestimmen Trypsinabhängigkeit

Die zytopathische Expression und Plaquebildung verschiedener Stämme von enteropathogenen bovinen Coronaviren wurden in einer Reihe von Zelltypen mit und ohne Trypsin analysiert. Der zelladaptierte, ursprünglich von MEBUS (1973) isolierte Stamm L 9 und 5 Wildtypstämme vermehrten sich in menschlichen Rektaltumorzellen. Sie verursachten zytopathische Veränderungen mit Zellfusion, was in der Gegenwart von Trypsin verstärkt auftrat. Der Stamm L 9 vermehrte sich ohne zytopathische Veränderungen in D 2 bovinen fötalen Milzzellen. Dabei hing die Aktivierung von Zellfusion ganz von der Gegenwart des Trypsins ab. Diese bovinen Milzzellen waren für alle Wildtypstämme des bovinen Coronavirus nicht empfänglich, auch nicht in Gegenwart von Trypsin. Der Stamm L 9 bildete Plaques in HRT-18-Zellklonen, jedoch hingen Plaquebildung und Morphologie von den Wirtszellklonen ab. Die Vermehrung bei Wildtypstämmen des bovinen Coronavirus hing ebenfalls von den Zellklonen ab, wobei die Plaquemorphologie zu unterschiedlichem Ausmaß von Trypsin beeinflußt wurde. Demnach bestimmen der jeweilige bovine Coronavirusstamm und die Wirtszelle die Trypsinabhängigkeit der zytopathischen Expression, der Zellfusion und der Plaquebildung.

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