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Respective stability of rotavirus and coronavirus in bovine milk

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Summary

Survival or inactivation of human and animal viruses in food, water, wastes has been studied extensively.

In France, the most popular varieties of cheese, such as Camembert, Brie, Coulommiers are still prepared with raw milk, outside of heat-treated milks. These soft cheeses make up 31 % of all cheeses consumed. 354 000 tons were manufactured in 1985. Consumption is regularly increasing by 2 % each year.

We studied the stability of 2 bovine enteroviruses : rotavirus and coronavirus, suspended in raw and sterilized bovine pre-cheese milks. The conditions of temperature, pH, rennet action and sodium chloride concentration examined in this study are those used routinely in the manufacture of soft cheeses.

The 2 viruses have a remarkable stability to low temperatures, acid pH, calf rennet and sodium chloride. The results are suggestive of a possible viral persistence in soft cheeses prepared with raw or heat-treated bovine milks.

Key words : Cheese - Rotavirus - Conavirus - Stability.

Résumé

Stabilité du rotavirus et du coronavirus dans le lait de vache

La survie ou l'inactivation des virus humains et animaux dans les aliments, l'eau, les effluents, a été beaucoup étudiée.

En France, les variétés les plus populaires de fromage, comme le Camembert, le Brie, le Coulommiers sont toujours préparées à partir de laits crus, en dehors de laits thermisés. Ces fromages à pâte molle correspondent à 31 % de la consommation annuelle. 354 000 tonnes ont été fabriquées en 1985. Cette consommation est en augmentation régulière de 2 % par an.

Nous avons étudié la stabilité de deux entérovirus bovins : rotavirus et coronavirus, mis en suspension dans des laits crus et stérilisés de vache. Les conditions de

température, pH, action de la présure et concentration en chlorure de sodium examinées dans cette étude sont celles qui interviennent couramment dans la fabrication des fromages à pâte molle.

Les deux virus ont une remarquable stabilité à basse température, aux pH acides, en présence de présure et de chlorure de sodium. Les résultats suggèrent une persistance virale possible dans les fromages à pâte molle fabriqués à partir de laits crus ou thermisés de vache.

Mots clés : Fromage - Rotavirus - Coronavirus - Stabilité.

Introduction

Outbreaks of human diseases due to bacterial (*Listeria*, *Yersinia*, *Campylobacter*, *Salmonella*...) or viral (Poliomyelitis Hepatitis A, Norwalk) contaminants, suggest that water, meat, milk and dairy products, are good vehicles for the transmission of these contaminants (WAIT and SOBSEY, 1983 ; HERRMANN and CLIVER, 1968 ; BARRETT, 1986). Also, investigations on the persistence of Aujeszky's disease virus, Teschen virus, Swine vesicular disease virus, Bovine leukemia virus (SULLIVAN *et al.*, 1971 ; LABIE, 1982 ; ELOIT *et al.*, 1986 ; LITTLEWOOD *et al.*, 1984), Foot and Mouth disease virus (SELLERS, 1969 ; BLACKWELL and HYDE, 1976), in meat, ham, or milk, have been reported in the literature.

Usually, we differentiate between primary and secondary contamination of foods. Primary contamination implies that the virus is present in the raw material before harvesting the finished product. Secondary contamination (direct or indirect) takes place at any time during processing, storage or distribution until the food is eaten. It may be due to human enteric viruses. Survival or inactivation of human or animal viruses in food, milk and dairy products, waters, wastes has been extensively studied (CLIVER *et al.*, 1970 ; BAUMGARTENER *et al.*, 1976 ; HERRMANN *et al.*, 1985 ; WARD *et al.*, 1986 ; TRAUB *et al.*, 1986).

Results of thermal inactivation trials of human enteroviruses demonstrated viral persistence in milk (KAPLAN and MELNICK, 1954), medium (WHITE *et al.*, 1982), or selective organic molecules (MILO, 1971). Also, CLIVER (1973) studied survival of human enteroviruses during processing of Cheddar cheese, and BLACKWELL (1976) studied survival of FMD virus in cheese.

In France, the most popular varieties of cheese are still prepared with raw milk. These soft cheeses make up 31 % of consumption equivalent to 354 000 T in 1985. Consumption is regularly increasing by 2 % each year (CNIEL, 1986). We were interested in the persistence of 2 bovine enteroviruses, rotavirus and coronavirus, after experimental contamination of milk samples, acid whey fractions, and media.

We studied the survival and infectivity of our 2 bovine viruses during the different steps of cheese making. The conditions examined in this study are those used routinely in the manufacture of soft cheeses : temperature of milk during storage (4 °C), rennet action and curd formation at 33 °C, presence of

low (5 %) and high (33 %) concentration of sodium chloride for brine preparation. We also studied thermal stability of both viruses at 13 °C, 20 °C, after heat-treatment at 63 °C, 72 °C and 120 °C, and in the pH range 4.6 – 6.3.

These experimental conditions are only models to understand the influence of different parameters on the survival or inactivation of bovine enteroviruses (and probably other closely related strains), throughout cheesemaking process.

I. Material and methods

A. Cells

Rhesus monkey kidneys (MA 104) cells were supplied by J. COHEN, I.N.R.A., Thiverval-Grignon. Cultures were made in 75 cm² or 150 cm² plastic flasks. The growth medium was Eagle's minimum essential medium, EMEM, containing 10 % fetal calf serum (FCS), 0.22 g/l sodium bicarbonate, 0.29 g/l glutamine, 100 U/ml penicillin, 100 µg/ml streptomycin, buffered with 20 mM HEPES (N – 2 hydroxyethylpiperazine – N' – 2 – ethanesulfonic acid). The maintenance medium was EMEM containing 2 % FCS. Monolayers were harvested with a mixture of trypsin (0.25 %) and versene (0.5 %).

Human rectal tumor (HRT 18) cells were supplied by J. LAPORTE *et al.* (1980). Cultures were made in 75 cm² or 150 cm² plastic flasks. The growth medium was RPMI 1640, containing 15 % FCS, 0.22 g/l sodium bicarbonate, 0.29 g/l glutamine, 5 mg/l tylosine, 180 mg/l lincocyne, buffered with 20 mM HEPES. The maintenance medium was RPMI 1640 supplemented by 2 % FCS. Monolayers were harvested with a mixture of trypsin (0.25 %) and versene (0.5 %).

B. Preparation of virus stocks

The bovine rotavirus, strain RF 45, was kindly supplied by J. COHEN. Briefly, the MA 104 monolayers were washed by MEM and incubated for 3 h at 37 °C. The rotavirus strain was inoculated at a low multiplicity of infection (MOI) : 0.1 plaque forming unit/cell (PFU) ; after 1 h adsorption, MEM containing 10 µg/ml trypsin, and 0.16 % tryptose phosphate broth was added. After complete cytopathic effect (CPE), cells were disrupted by three cycles of freezing – thawing. The viral suspension was centrifuged at 1 500 rpm for 15 min at 4 °C, then the supernatant was filtered through a 0.22 µm Millipore membrane. The virus stock was stored in 1 ml tubes, at – 20 °C. The final titer was : 4×10^9 PFU/ml.

The bovine coronavirus, strain G 110, was kindly supplied by J. LAPORTE. The HRT 18 monolayers were washed with RPMI 1640. The coronavirus strain was inoculated at a MOI : 0.1 PFU/cell. After 1 h adsorption, the maintenance medium was added to the cells. After complete CPE, cells were disrupted by one cycle of freezing – thawing. The viral suspension was centrifuged at 1 500 rpm for 15 min at 4 °C, then the supernatant filtered through a 0.22 µm Millipore membrane. The virus stock was stored in 1 ml tubes, at – 20 °C. The final titer was 2×10^9 PFU/ml.

Milk samples : Whole raw milk was obtained from cows maintained at a university farm.

Heat-treated milks : sterilized, UHT, and pasteurized milks, were bought at a supermarket. Twenty ml samples were used in this study.

Whey fractions : were extracted from milks either by acid precipitation (pH 4.6) or rennet coagulation (pH 6.3) (ALAIS, 1984).

pH : pH of sterilized milk and media samples were adjusted to pH 4.6, 5.4 and 6.3 with N HCl. Twenty ml samples were contaminated by 2×10^5 PFU. Heat treatment was done for 2 h at 33 °C. pH 4.6 is acid whey pH, pH 5.4 is curd pH, pH 6.3 is pH for rennet treatment.

NaCl : sterilized milk and media samples were treated with 5 % and 33 % NaCl (representative of brine solution) then contaminated by 10^6 PFU/ml. Incubation time was 7 d at 13 °C.

Rennet treatment : sterilized milk and media samples were treated with commercial rennet (Coopération Pharmaceutique Française, 44000 Nantes : chymosine activity > 50 mg/l). Final concentration was 12.5 µg for 100 ml sample, in presence of CaCl₂ : 0.5 g/l, pH 6.3. Twenty ml samples were contaminated with 5 log₁₀ PFU/ml, then distributed in one ml aliquots. The incubation time was 7 h at 33 °C.

Temperature action : we studied the thermal inactivation of bovine rotavirus and coronavirus suspended in raw milk, heat-treated milks and corresponding wheys. Twenty ml samples were inoculated then distributed in one ml aliquots. Final concentration of virus in milk and whey samples was 6.8 log₁₀ PFU/ml. Varying temperatures and times were tested : 4 °C for 13 d, 20 °C for 10 d. Heat treatments were done in a water-bath, at 63 °C for 1 h and at 72 °C for 1 h. Contaminated samples were also heat treated at 120 °C for 15 min, in an autoclave.

Sample inoculations : we made direct inoculation on specific tissue culture. Before plaque assay, we treated 2 × 1 ml of each rotavirus sample with 10 µg/ml trypsin solution for 1 h at 37 °C. All rotavirus and coronavirus samples were treated with a mixture of antibiotics : 10⁴IU/ml penicillin, 10 mg/ml streptomycin, 2 µg/ml amphotericin B, and 750 µg/ml neomycin sulfate. The incubation was 1 h at 37 °C for rotavirus samples and overnight at 4 °C for the coronavirus samples. Antibiotic concentrations used in raw milk and whey samples provided necessary bactericidal activity.

C. Viral plaque forming unit (PFU) assay

1. Rotavirus

The protocol has been described by SCHERRER *et al.* (1976). Confluent monolayers of MA104 cells, 4 days old, were prepared in disposable tissue culture plates (Greiner, 6 wells). After 3 washes with phosphate buffered saline (PBS), samples were inoculated onto monolayer and held for 1.5 h at 37 °C in a CO₂ incubator. Inoculum was removed and agarose overlay added. After 3 days, an agarose plus neutral red overlay was added. Plaques were counted after 3 to 5 days.

2. Coronavirus

The protocol has been described by VAUTHEROT (1981). Confluent monolayers of HRT 18 cells, 4 days old, were prepared in disposable tissue culture plates (Greiner, 6 wells). After 2 washes with PBS, samples were inoculated onto monolayer and held for 1.5 h, at 37 °C in a CO₂ incubator. Inoculum was removed and agarose overlay added. After 2 days of incubation, the overlay was poured off. One ml of 2 % rat red blood cell suspension was added, and left for 30 min at 37 °C. After a PBS wash, the hemadsorption plaques were counted.

II. Results and discussion

We first studied the rotavirus recovery from milk and the acid whey derivative inoculated with 1.5×10^4 PFU/ml. Previous heat treatment of milk was pasteurization, sterilization and UHT (table 1). Approximately 93 % of virus content was recovered from all heat treated milk and acid whey samples.

TABLE 1

Rotavirus recovery from milk and acid wheys samples inoculated with 1.5×10^4 PFU/ml, as a function of previous heat treatment of milks

Pourcentages de récupération du rotavirus après contamination d'échantillons de lait et de lactosérum acide en fonction du traitement thermique antérieur. Inoculum de départ $1,5 \times 10^4$ PFU/ml

Milk and wheys samples	Virus recovery (PFU/sample)	%
Whole raw milk	0	0
acid whey	0	0
Whole pasteurized milk	1.36×10^4	90
acid whey	1.44×10^4	96
Whole sterilized milk	1.36×10^4	90
acid whey	1.3×10^4	86
Whole UHT milk	1.36×10^4	90
acid whey	1.40×10^4	93

TABLE 2

Coronavirus, virus recovery from milk samples inoculated with 3.3×10^4 PFU/ml, as a function of previous heat treatment of milks

Pourcentages de récupération du Coronavirus après contamination d'échantillons de lait en fonction du traitement thermique antérieur. Inoculum de départ $3,3 \times 10^4$ PFU/ml

Milk samples	Virus recovery PFU/sample	%
Whole raw milk	0	0
Whole pasteurized milk	1.9×10^4	56
Whole sterilized milk	2×10^4	60
Whole UHT milk	1.2×10^4	36

With raw milk, we observed an antirotavirus effect. The rate of inactivation was 100 %. Similar results were obtained in heated milk and whey samples artificially contaminated with coronavirus (table 2). Virus recovery was between 40 % and 60 % in heat treated milk samples. But the viricidal activity of whole raw milk against coronavirus was quite significant.

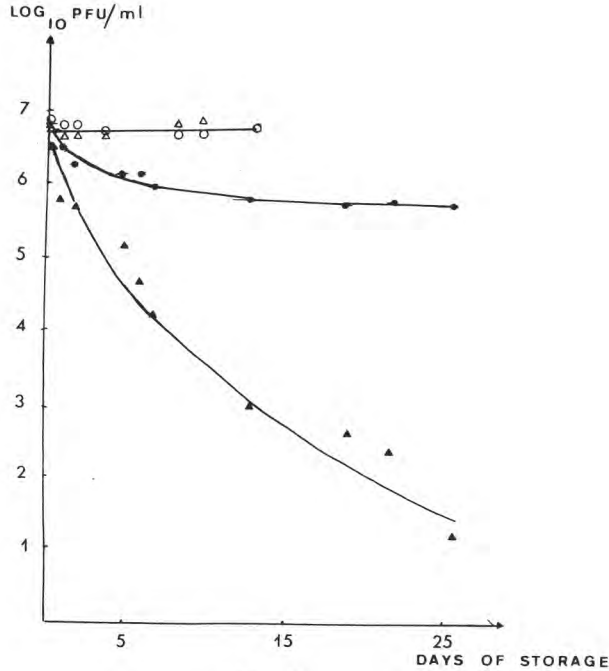


Fig. 1

Effect of storage of whey samples (sterilized bovine milk) contaminated by bovine rotavirus at 4 °C : ○-○ ; 20 °C : △-△ and bovine coronavirus at 4 °C : ●-● ; 20 °C : ▲-▲, on infectivity. Log₁₀ PFU/ml.

Action de la conservation des échantillons de lactosérum (lait stérilisé de vache) contaminés par le rotavirus bovin à 4 °C : ○-○ ; 20 °C : △-△ et du coronavirus bovin à 4 °C : ●-● ; 20 °C : ▲-▲ sur leur infectivité. Log₁₀ PFU/ml.

In further experiments we studied the temporal inactivation of bovine rotavirus and coronavirus suspended in acid whey samples prepared from raw milk, and sterilized milk (tables 3, 4, 5 ; fig. 1). Thermal inactivation studies at 4 °C, 20 °C, 63 °C, and 120 °C confirm relative stability of bovine rotavirus in sterilized milk, the acid whey derivative or MEM, when incubated at 4 °C, 20 °C for 13 d and 10 d. After heat treatment at 63 °C and 72 °C, rotavirus was inactivated within 20 min and 5 min respectively. Under the same experimental conditions bovine coronavirus was less stable. After incubation at 4 °C for 26 d, and 20 °C for 26 d, we observed a loss of one log₁₀ PFU/ml and 5 log₁₀ PFU/ml respectively. After heat treatment at 63 °C and 72 °C,

coronavirus was inactivated within 5 min and 15 s, respectively. After heat treatment at 120 °C, no rotavirus or coronavirus infectivity was detected after 5 min, 10 min and 15 min. In the same experimental conditions, we were unable to detect viruses in raw milk samples.

TABLE 3

Rotavirus recovery from acid wheys inoculated (raw and sterilized milks) as a function of temperature and incubation time. Log₁₀ PFU/ml. Virus input : 6.8 log₁₀ PFU/ml

Pourcentages de récupération du rotavirus après contamination de lactosérum acide de lait cru et stérilisé, en fonction de la température et du temps d'incubation.

Log₁₀ PFU/ml - Inoculum de départ 6,8 log₁₀ PFU/ml

(a) pas de virus (b) non testé

Days of storage	4 °C		20 °C	
	Raw milk	Sterilized milk	Raw milk	Sterilized milk
0	— (a)	6.8	—	6.73
1	—	6.8	—	6.71
2	—	6.77	—	6.71
3	—	6.74	—	6.71
8	—	6.71	—	6.71
10	—	6.68	—	6.71
13	—	6.8	ND (b)	ND

Time of incubation (minutes)	63 °C		Time of incubation (secondes and minutes)	72 °C	
	Raw milk	Sterilized milk		Raw milk	Sterilized milk
0	— (a)	5.71	0	—	ND
5	—	1.6	15 s	—	6.88
10	—	1.6	30 s	—	6.83
15	—	0.9	1 min	—	6.77
20	—	—	5	—	—
30	—	—	15	—	—
45	—	—	30	—	—
60	—	—	60	—	—

(a) no virus isolated.

(b) not done.

TABLE 4

Coronavirus recovery from acid wheys inoculated (raw and sterilized milks) as a function of temperature and incubation time. Log₁₀ PFU/ml. Virus input : 6.8 log₁₀ PFU/ml

Pourcentages de récupération de coronavirus après contamination de lactosérum acide de lait cru et de lait stérilisé, en fonction de la température et du temps d'incubation.

Log₁₀ PFU/ml - Inoculum de départ 6,8 log₁₀ PFU/ml

(a) pas de virus

Days of storage	4 °C		20 °C	
	Raw milk	Sterilized milk	Raw milk	Sterilized milk
0	2.39	6.87	2.17	6.87
1	— (a)	6.57	—	5.81
2	—	6.27	—	5.71
5	—	6.14	—	5.17
6	—	6.07	—	4.64
7	—	6.04	—	4.25
13	—	5.77	—	2.95
19	—	5.71	—	2.56
22	—	5.69	—	2.04
26	—	5.70	—	1.55

Time of incubation (minutes)	63 °C		Time of incubation (secondes and minutes)	72 °C	
	Raw milk	Sterilized milk		Raw milk	Sterilized milk
0	— (a)	4.2	0	—	6.87
5	—	—	15 s	—	—
10	—	—	30 s	—	—
15	—	—	1 min	—	—
20	—	—	5	—	—
30	—	—	15	—	—
45	—	—	30	—	—
60	—	—	60	—	—

(a) no virus isolated.

TABLE 5

Rotavirus and coronavirus recovery from acid wheys inoculated (raw and sterilized milks) after treatment at 120 °C as a function of time. Virus input 6.8 log₁₀ PFU/ml
Infectivité réiduelle après contamination de lactosérum acide de lait cru et de lait stérilisé par le rotavirus et le coronavirus en fonction du temps de traitement à 120 °C.
Inoculum de départ 6,8 log₁₀ PFU/ml
 (a) pas de virus

Time (min)	Rotavirus		Coronavirus	
	Raw milk	Sterilized milk	Raw milk	Sterilized milk
5	— (a)	—	—	—
10	—	—	—	—
15	—	—	—	—

(a) no virus isolated.

TABLE 6

Rotavirus and coronavirus recovery from sterilized milk and media samples at 4 °C as a function of days of storage. Log₁₀ PFU/ml
Infectivité des échantillons de lait stérilisé et de milieux contaminés par le rotavirus et le coronavirus, à 4 °C en fonction des jours d'incubation. Log₁₀ PFU/ml

Days of storage	Rotavirus		Coronavirus	
	Milk	MEM	Milk	RPMI
T = 0	4.51	4.07	3.98	3.66
T = 1	4.47	3.94	3.63	3.23
T = 2	ND (a)	ND	3.47	2.90
T = 3	4.49	3.55	3.59	2.60
T = 6	ND	ND	3.68	2.69
T = 7	4.51	3.39	3.57	2.47

(a) not done.

The thermal stability of bovine viruses was further studied in sterilized milk, MEM or RPMI 1640 media, at 4 °C, for 7 days (table 6, fig. 2). Both rotavirus and coronavirus were stable in sterilized milk preparations held at 4 °C. But, when suspended in medium, these viruses lost 0.6 log₁₀ PFU/ml and 1.2 log₁₀ PFU/ml of infectivity after 7 days of incubation respectively.

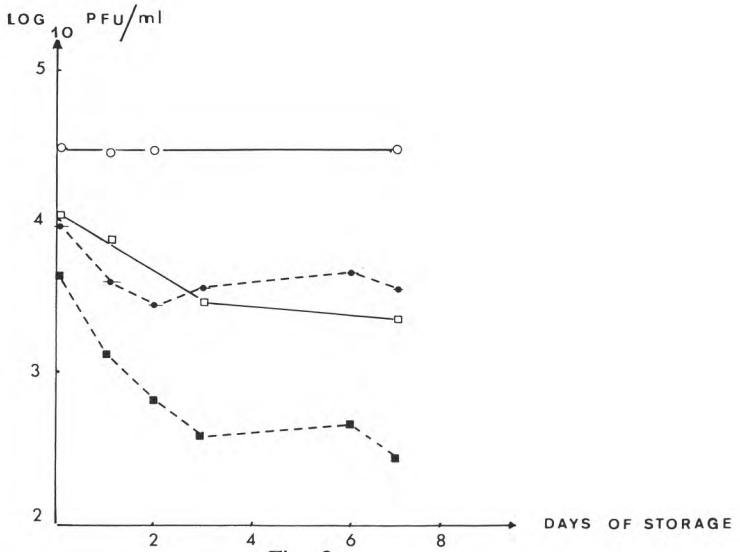


Fig. 2

Effect of storage at 4 °C on infectivity (\log_{10} PFU/ml) of bovine rotavirus suspended in MEM □-□, or sterilized bovine milk ○-○, and of bovine coronavirus suspended in RPMI 1640 ■-■, or sterilized bovine milk ●-●.

Etude de l'infectivité (\log_{10} PFU/ml) du rotavirus bovin suspendu en MEM □-□, ou du lait stérilisé ○-○, et de celle du coronavirus bovin suspendu en RPMI 1640 ■-■, ou du lait stérilisé ●-●, en fonction de l'incubation à 4 °C.

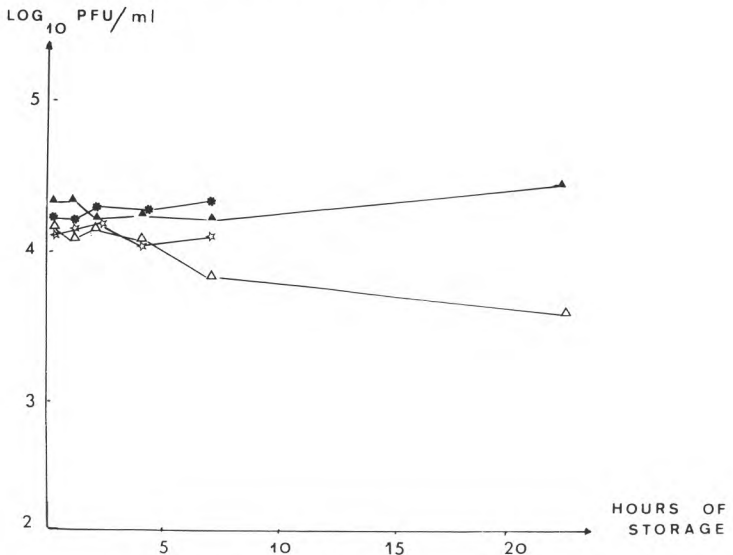


Fig. 3

Effect of incubation at 33 °C on infectivity (\log_{10} PFU/ml) of bovine rotavirus suspended in MEM △-△, MEM supplemented with rennet ☆-☆, sterilized bovine milk ▲-▲, and sterilized bovine milk supplemented with rennet ★-★.

Effet de l'incubation à 33 °C sur l'infectivité (\log_{10} PFU/ml) du rotavirus bovin suspendu en MEM △-△, MEM + présure ☆-☆, en lait stérilisé ▲-▲, et lait stérilisé + présure ★-★.

Bovine rotavirus infectivity was not affected after 2 h incubation at 33 °C at pH 4.6, 5.4, and 6.3, respectively (table 7). Rotavirus was more acid stable than bovine coronavirus. When suspended in RPMI 1640 medium, coronavirus infectivity decreased 1.4 and 0.3 log₁₀ PFU/ml respectively at pH 4.6 and 5.4 respectively.

During 7 h of incubation at 33 °C, the presence of calf rennet had no specific effect on rotavirus (table 8, fig. 3) and coronavirus infectivity (table 9, fig. 4). Due to thermal inactivation, control rotavirus titer decreased 0.6 log₁₀ PFU/ml when suspended in MEM, and coronavirus 2.6 and 2 log₁₀ PFU/ml, respectively when suspended in RPMI 1640 medium and milk respectively. Concentration of calf rennet (12.5 µg/ml) sufficient for milk clotting had no effect on viral infectivity.

TABLE 7

Rotavirus and coronavirus recovery from sterilized milk and media samples as a function of pH. Incubation 2 h at 37 °C. Log₁₀ PFU/ml

Infectivité des échantillons de lait stérilisé et de milieux contaminés par le rotavirus et le coronavirus en fonction de 3 pH. Incubation 2 h à 37 °C. Log₁₀ PFU/ml

pH	Rotavirus		Coronavirus	
	Milk	MEM	Milk	RPMI
6.3	4.41	4.24	3.82	3.69
5.4	4.39	4.25	3.74	3.34
4.6	> 4.30	> 4.30	3.75	2.30

TABLE 8

Kinetic of inactivation of rotavirus in sterilized milk and MEM medium, at 33 °C as a function of incubation time, and presence of rennet. Log₁₀ PFU/ml

Cinétique d'inactivation du rotavirus en suspension dans du lait stérilisé et du milieu MEM à 33 °C en fonction du temps d'incubation et de la présence de présure. Log₁₀ PFU/ml

Hours of incubation	33 °C (control)		33 °C + rennet	
	Milk	MEM	Milk	MEM
T = 0	4.37	4.23	4.25	4.13
T = 1	4.36	4.11	4.20	4.17
T = 2	4.24	4.17	4.27	4.17
T = 4	4.27	4.07	4.27	4.04
T = 7	4.25	3.88	4.34	4.11
T = 22	4.32	3.64	ND (a)	ND

(a) not done.

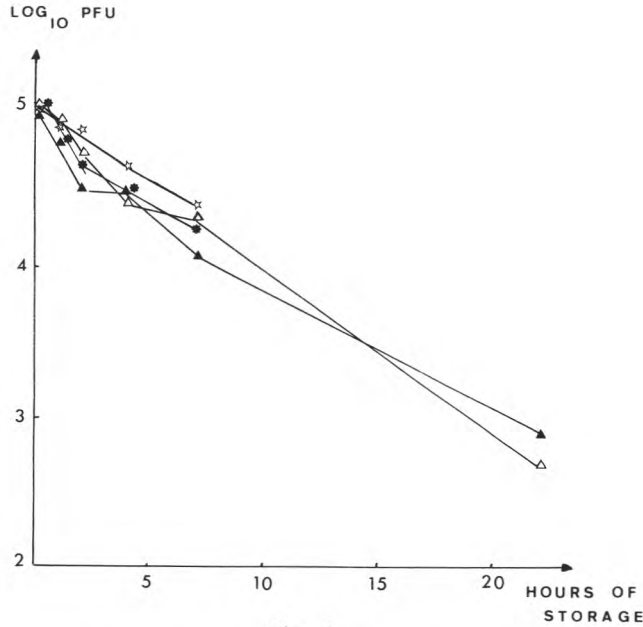


Fig. 4

Effect of incubation at 33 °C on infectivity (\log_{10} PFU/ml) of bovine coronavirus suspended in RPMI 1640 \triangle - \triangle , RPMI 1640 supplemented with rennet \star - \star , in sterilized bovine milk \blacktriangle - \blacktriangle , and sterilized bovine milk supplemented with rennet \blackstar - \blackstar .

Effet de l'incubation à 33 °C sur l'inféctivité (\log_{10} PFU/ml) du coronavirus bovin suspendu en RPMI 1640 \triangle - \triangle , RPMI 1640 + présure \star - \star , en lait stérilisé \blacktriangle - \blacktriangle , et lait stérilisé + présure \blackstar - \blackstar .

TABLE 9

Kinetic of inactivation of coronavirus in sterilized milk and RPMI 1640 medium at 33 °C, as a function of incubation time and presence of rennet. \log_{10} PFU/ml

Cinétique d'inactivation du coronavirus en suspension dans du lait stérilisé et du milieu RPMI 1640, à 33 °C en fonction du temps d'incubation et de la présence de présure. \log_{10} PFU/ml

Hours of incubation	33 °C (control)		33 °C + rennet	
	Milk	RPMI	Milk	RPMI
T = 0	5.	5.07	5.07	5.04
T = 1	4.82	4.96	4.82	4.95
T = 2	4.49	4.77	4.66	4.91
T = 4	4.27	4.47	4.52	4.74
T = 7	4.11	4.36	4.3	4.51
T = 22	2.93	2.47	ND (a)	ND

(a) not done.

TABLE 10

Kinetic of inactivation of rotavirus and coronavirus in sterilized milk and MEM or RPMI 1640 media, at 13 °C, as a function of incubation time and presence of NaCl. Log₁₀ PFU/ml

Cinétique d'inactivation du rotavirus et du coronavirus en suspension dans du lait stérilisé et les milieux MEM ou RPMI 1640, à 13 °C en fonction du temps d'incubation et de la présence de NaCl. Log₁₀ PFU/ml

13 °C (control)

Days of storage	Rotavirus		Coronavirus	
	Milk	MEM	Milk	RPMI
T = 0	6.49	6.03	5.55	5.74
T = 1	6.41	6.01	5.25	5.34
T = 3	6.38	4.64	4.83	5.
T = 7	6.50	4.80	4.41	4.34

13 °C + 5 % NaCl

Days of storage	Rotavirus		Coronavirus	
	Milk	MEM	Milk	RPMI
T = 0	6.48	5.91	6.17	6.11
T = 1	6.50	4.85	6.23	5.97
T = 3	6.55	5.04	5.69	5.51
T = 7	6.55	4.98	4.84	4.79

13 °C + 33 % NaCl

Days of storage	Rotavirus		Coronavirus	
	Milk	MEM	Milk	RPMI
T = 0	5.92	4.68	6.04	4.86
T = 1	6.19	4.50	5.72	4.76
T = 3	6.20	4.38	5.34	4.38
T = 7	6.0	3.90	4.76	> 2.55

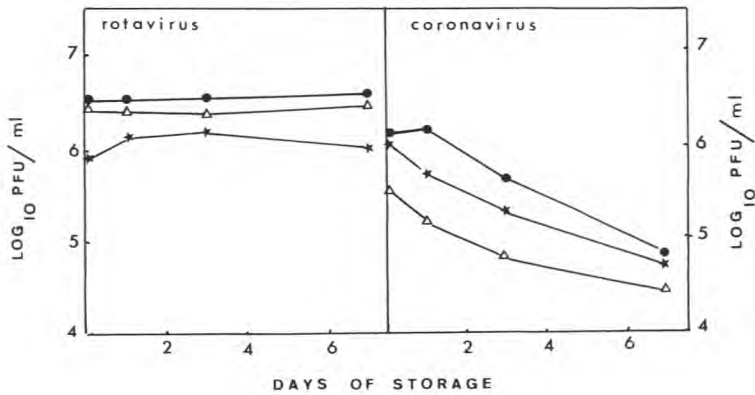


Fig. 5

Effect of NaCl treatment at 13 °C on the infectivity of bovine rotavirus and coronavirus suspended in sterilized bovine milk supplemented with 5 % ●—●, or 33 % ★—★ NaCl. Control without NaCl △—△.

Action du NaCl à 13 °C sur l'infectivité du rotavirus et coronavirus bovins en suspension dans du lait stérilisé + 5 % NaCl ●—●, ou 33 % NaCl ★—★. Contrôle sans NaCl △—△.

After 7 days of incubation, at 13 °C, control rotavirus suspended in MEM lost 1.2 log₁₀ PFU/ml of infectivity (table 10, fig. 5). The addition of 5 % or 33 % NaCl, had no significant virucidal effect, when virus was suspended in milk or MEM respectively. Bovine coronavirus was equally stable in milk or medium with 5 % NaCl, and lost 1.3 log₁₀ PFU/ml of infectivity. When coronavirus was suspended in RPMI 1640 with 33 % NaCl, the loss of infectivity was about 2.3 log₁₀ PFU/ml. Rotavirus was more stable to high concentration of sodium chloride when suspended in MEM, than coronavirus.

When compared to media samples, both viruses were more stable in sterilized milk samples kept for 13 d and 26 d at 4 °C, 7 d at 13 °C or 1 d at 33 °C. But, after long storage of 13 d and 26 d respectively at 20 °C, bovine coronavirus lost 4 log₁₀ PFU/ml and greater than 5 log₁₀ PFU/ml of infectivity respectively.

Results concerning pasteurization treatment of bovine rotavirus and coronavirus were in agreement with our knowledge on thermal inactivation of these viruses. Other factors routinely used in the manufacture of soft cheeses, such as acid pH, rennet and sodium chloride, have in fact no action on rotavirus infectivity, but a more significant action on coronavirus infectivity.

We observed a strong antiviral activity in raw milk. This observation was elucidated in a previous paper (PANON *et al.*, 1987). This activity seems due to antibody like substances with neutralizing activities. It could be responsible for inapparent primary or secondary contaminations. But on the other hand, microbial and proteolytic inactivation of anteroviruses has also been studied (CLIVER and HERRMANN, 1972 ; GILBERT *et al.*, 1983).

Quality assurance for raw milk in France has to be improved (VAN DEN BERG, 1986). Since we know the relatively good stability of bovine rotavirus and coronavirus suspended in sterilized milk to factors such as low temperature, acid pH, calf rennet or NaCl, persistence of the two viruses throughout cheese making process could exist. Based on results of study, soft cheese prepared from raw milk could be considered as a potentially good « vehicle » for the transmission of bovine enteroviruses, and probably also for human enteroviruses which are antigenically closely related to animal strains (WOODE *et al.*, 1976 ; WARD *et al.*, 1985). Because of the demonstrated stability of these viruses in heat-treated milk, a potential exists for « secondary » contamination of these products.

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