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Studies on Bovine Breda Virus

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ABSTRACT

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Diarrheic feces from 21 calves were examined by electron microscopy and 16 contained particles morphologically similar to those of Breda virus. The particles were spherical or elongated, 60-270 nm in greatest dimension and had surface spikes 9-13 nm long. Convalescent serum from a human patient with Breda virus-associated diarrhea reacted with one of the bovine viruses by immune electron microscopy, suggesting a serological resemblance between human and bovine Breda-like viruses. Immune electron-microscopy and immunofluorescence demonstrated that isolates of bovine Breda virus from the U.S.A. were related to the French virus. One of the viruses had a density in sucrose solution of 1.16, similar to the value for Berne virus.

INTRODUCTION

Rotaviruses and coronaviruses have been established as major viral agents of diarrhea in young animals such as calves and pigs (Pensaert and de Bouck, 1978). In human subjects rotaviruses are recognized as diarrheic agents, but the etiological role of enteric coronaviruses in human diarrhea has not been clearly demonstrated (Caul and Egglestone, 1982).

Recently, a new enveloped virus called Breda virus was isolated by Woode et al. (1982) from diarrheic calf feces in Iowa, U.S.A. Others report the presence of morphologically similar particles in human babies and adults suffering from gastroenteritis (Moscovici et al., 1980; Vaucher et al., 1982; Lamouliatte

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and du Pasquier, 1983; Beards et al., 1984; Horzinek and Weiss, 1984). Several years ago an unclassified enveloped virus, the Berne virus, was isolated from a horse with pseudomembranous enteritis possibly caused by a *Salmonella*; this virus was studied by Weiss et al. (1983) and shown to be different from coronaviruses (Horzinek et al., 1984) and antigenically related to Breda viruses (Woode et al., 1985). The Berne virus has been proposed by Horzinek and Weiss (1984) to represent the type of a new family, the Toroviridae, which could include the similar particles seen in human and bovine feces. For several years we have observed Breda-like particles in human gastroenteritis, and these particles could not be cultivated in routine laboratory cell culture such as monkey kidney cells or human diploïd cells. Because bovine Breda viruses might be more amenable to study, we have examined diarrheic calf feces for these agents. We are now able to specify the major role played by these viruses in the diarrhea of young calves, and to provide further information on the bovine Breda-like agent.

MATERIALS AND METHODS

Source of viruses

From May 1984 to April 1985, we received from the Services Departementaux du Lot et Garonne 22 calf fecal samples from various outbreaks of gastroenteritis which developed in several properties. Except for one normal specimen, the samples originated from animal suffering from mild diarrhea or hemorrhagic enteritis. Thirteen of the calves were battery reared and 9 were from traditional stock farms (Table I). Infectious agents other than viruses had been previously sought by the Veterinary Department (Table I). From some of the calves, acute and convalescent sera were obtained.

Electron microscopic examination (EM)

Direct diagnosis

Feces were diluted 1/5 in pH 7.2 phosphate-buffered saline (PBS) and centrifuged for 30 min at 6000×*g*. One volume of clarified fecal supernatant was added to an equal volume of Freon, then shaken mechanically and centrifuged for 20 min at 6000×*g*. One drop of the resulting aqueous phase was placed on a collodion–carbon-coated grid and stained with phosphotungstic acid (PTA, Merck) 2% at pH 6.8.

Microscopic examination was performed on an EM 301 Phillips microscope under 60 KV at a minimum magnification of 34 000. Size measurements of viral particles were made, using catalase crystals as standards.

Examination of cell-culture supernatants

The cell-culture supernatants were frozen and thawed 3 times and then clarified at 6000 $\times g$ for 15 min, the supernatants were concentrated 20-fold by ultracentrifugation at 65 000 $\times g$ in a Beckman "Airfuge" before being stained as described above.

Immune electron microscopy (IEM)

For this experiment, we used a fecal sample which contained numerous Breda-like particles on direct EM examination (Sample 736 (1)). This was successively filtered through 0.8-, 0.45- and 0.2-μm pore diameter membranes. One hundred microliters of the filtrate was mixed with an equal volume of one of the following: (i) 1/100 dilution in PBS of acute and convalescent homologous sera from calves (736 (1) and 736 (2)); (ii) 1/100 dilution in PBS of acute and convalescent human serum (Bar 1 and Bar 2) from an adult who presented Breda-like virus-associated diarrhea; (iii) 1/100 dilution in PBS of rabbit anti-bovine coronavirus serum. Bar 1 serum was previously shown not to react significantly with homologous human particles purified from feces, on the other hand serum Bar 2 reacted with the human particles. When tested against reference bovine Breda virus, Bar 1 serum did not react with Breda 1 serotype, but reacted slightly with Breda 2 serotype; Bar 2 serum reacted slightly with Breda 1 serotype and strongly with Breda 2 serotype (Beards et al., 1984).

After 1 h incubation in a wet chamber at 37°C and overnight at 4°C, the serum–virus mixtures were pelleted in a Beckman "Airfuge" at 30 000 $\times g$ for 10 min.

The resulting pellets were resuspended in 50 μl of distilled water and stained as described above.

Cell culture of virus

Human rectal tumor cells (HRT 18) were prepared in tubes and in LAB-TEK chambers (Miles Laboratories), using RPMI 1640 growth medium with 15% fetal calf serum (FCS). Prior to inoculation, the cells were washed twice with the medium devoid of serum. After 1 h incubation with nystatin (40 IU ml^{-1}), streptomycin (2 mg ml^{-1}), penicillin 200 (IU ml^{-1}), the fecal samples were diluted 1/5 in RPMI containing 2% FCS and 0.12 μg pancreatin ml^{-1} (Bohl et al., 1984). The tubes and Lab-Tek chambers were then inoculated. After 1 h incubation at 37°C, the inocula were removed and replaced by 2% FCS RPMI containing 0.12 μg pancreatin ml^{-1}.

The cultures were observed daily for the presence of cytopathic changes. The cell monolayers in the Lab-Tek chambers were fixed 24 h post-infection for immunofluorescence, and the tubes were used for hemadsorption.

TABLE I

Summary of the microbiological diagnosis

Sample	Virological diagnosis		Other pathogens	Clinical findings	Mortality[b]	Age in weeks	Breeding mode
	EM[a]	ELISA (rotavirus)					
736 (1)	Breda virus + + +	–	–	Hemorrhagic enteritis and hepatitis	+ +	4-8 W	Battery[c]
736 (2)	Breda virus +	–	–	Hemorrhagic enteritis and hepatitis	+ +	4-8 W	Battery
736 (3)	Breda virus +	–	–	Hemorrhagic enteritis and hepatitis	+ +	4-8W	Battery
651 (1)	Breda virus + +	–	–	Hemorrhagic enteritis	NK	4-8 W	Battery
651 (2)	Breda virus +	–	–	Hemorrhagic enteritis	NK	4-8 W	Battery
656 (3)	Breda virus +	–	–	Hemorrhagic enteritis	NK	4-8 W	Battery
1017 (1)	Breda virus + +	–	–	Diarrhea, no autopsy	+	0-4 W	Traditional
92 (2)	Breda virus +	–	–	Diarrhea, no autopsy	+	0-4 W	Traditional
92 (3)	Breda virus +	–	Cryptosporidiae	Diarrhea, no autopsy	+	0-4 W	Traditional
92 (4)	Breda virus +	–	–	Diarrhea, no autopsy	+	0-4 W	Traditional
197 (1)	Breda virus + +	–	Cryptosporidiae, *Escherichia coli*	Diarrhea	+	0-4 W	Traditional

TABLE I (*continued*)

241 (1)	Breda virus (+)	–	–	Diarrhea and severe dehydration	++	0-4 W	Battery
241 (2)	Breda virus++	–	–	Diarrhea and severe dehydration	++	0-3 W	Battery
386 (1)	Breda virus++		–	Diarrhea	–	0-3 W	Traditional
	Rotavirus+	+					
386 (2)	Breda virus+	+		Diarrhea	–	0-3 W	Traditional
	Rotavirus+		–				
92 (1)	Breda virus++		–	Diarrhea, no autopsy	+	0-4 W	Traditional
	Rotavirus+	+					
461 (1)	Coronavirus (+)	–	–	Hemorrhagic enteritis	+	0-4 W	Traditional
241 (3)	Coronavirus (+)	–	–	Diarrhea and severe dehydration	++		Battery
419 (1)	Rotavirus+	+	–	Hemorrhagic enteritis and hepatitis	+	4-8 W	Battery
656 (4)	Rotavirus (+)	–	–	Healthy	–	4-8 W	Battery
556 (1)	Rotavirus+	+	–	Diarrhea	–	4-8 W	Battery
870 (1)	No virus	–	*S. typhimurium*	Hemorrhagic enteritis	++	4-8 W	Battery

[a] (+) <5 particles per 20 squares; + 0-5 particles per square; ++ 5-10 particles per square; +++ >10 particles per square.
[b] – no mortality; + low mortality 2-5%; ++ high mortality 10-15%; NK not known.
[c] Battery: rearing in stables of 100 animals, each of them being kept in an individual stall.

Immunofluorescence (IF)

After rinsing with PBS, pH 7.2, the cell monolayers were fixed with methanol at room temperature for 10 min, and if the IF reaction was delayed they were stored at −80°C. Inoculated and control cells were allowed to react separately with one of the following: (i) monoclonal anti-bovine coronavirus A 912 Ab (provided by J. Laporte); (ii) bovine convalescent serum from 736 (1) calf (this serum was previously adsorbed with bovine rotavirus concentrated from a MK 2 cell culture supernatant); (iii) rabbit anti-bovine rotavirus serum produced in our laboratory (Lamouliatte-Jambon, 1982). The next step was to apply antisera which were conjugated with fluorescein isothiocyanate (anti-mouse and anti-rabbit from Nordic Lab., anti-bovine from Wellcome Lab.).

Hemagglutination (HA) - hemagglutination inhibition (HAI) - hemadsorption (HAD)

This test was performed according to the method described by Woode et al. (1982) using V-bottomed microtiter plates. Serial 2-fold dilutions of the fecal samples diluted 1/5 in PBS and centrifuged at $6000 \times g$ were made in 25 µl of PBS. Fetal calf serum diluted 1/5 was adsorbed for 1 h at 4°C with packed rat erythrocytes, then 25 µl of a 1/40 dilution of this serum was added to each well. Twenty five microliters of 1% rat erythrocytes in PBS with 0.1% bovine serum albumin were added to each well and the plates were incubated for 2 h at room temperature. Bovine coronavirus was used as positive control.

Convalescent sera from two Breda virus-positive calves (736 (1) Ab and 92 (4) Ab) and rabbit anti-bovine coronavirus antiserum (BCV F 15 Ab), which had been adsorbed with rat erythrocytes and diluted 1/40 in PBS, were used in the test in place of fetal calf serum; the purpose of this procedure was to obtain inhibition of hemagglutination and thus to attempt to identify the antigenic character of the hemagglutinating fecal particles. HAI was performed only on samples with HA titers $\geqslant 1/16$.

Hemadsorption was carried out on the fourth day post-infection. The medium of inoculated and control tubes was removed and replaced by 1 ml of a 0.2% suspension of fresh rat erythrocytes in pH 7.2 PBS. The tubes were incubated for 1 h at room temperature, and then observed on a light microscope at a magnification of $\times 100$.

Purification on sucrose gradient and buoyant density determination

This purification was run in parallel with a cell culture-adapted bovine coronavirus (strain BCV F 15) according to the method of Laporte and Bobulesco (1981). The Breda virus used for this process was the Freon-treated,

filtered 736 (1) fecal supernatant and BCV was obtained from infected HRT 18 cells (Laporte et al., 1979).

One hundred and twenty milliliters of 736 (1) sample and 90 ml of clarified supernatant of BCV-infected cells were ultracentrifuged for 60 min at 95 800×*g* in a 45 Ti rotor (Beckman). The pellets were resuspended in 3 ml of distilled water and stored overnight at 4°C. On the following day, each concentrated viral suspension was layered onto 40 ml of a 20–45% w/w sucrose gradient in distilled water and centrifuged for 120 min at 82 500×*g* in a swinging-bucket rotor SW 27 (Beckman). The viral bands were collected, diluted in distilled water and centrifuged for 60 min at 95 800×*g* in the 45 Ti rotor.

Each resulting pellet was diluted in 2 ml of bi-distilled water, layered onto a 20–60% w/w sucrose gradient and centrifuged for 18 h at 82 500×*g* in the SW 27 rotor.

All centrifugation steps were performed at 4°C.

Rotavirus detection by ELISA

The samples were diluted 1/5 in PBS (pH 7.2), clarified at 6000×*g*, and then submitted to ELISA according to a previously described method (Lamouliatte-Jambon, 1982). In brief, each sample was incubated for 90 min at 37°C in a microtiter plate coated with rabbit anti-bovine rotavirus antibodies. Anti-bovine rotavirus conjugate (peroxidase labelled) was added and left for 90 min at 37°C. The substrate was incubated for 15 min at room temperature, and the plates were then read on a Titertek Multiscan at 492 nm.

RESULTS

Presence of virus in feces

Of the 21 diarrheic samples, 13 contained Breda viruses in variable amount, three contained both Breda and rotaviruses, two contained coronaviruses, two were positive for rotaviruses by EM and ELISA and one did not contain any virus. *Salmonella typhimurium* was demonstrated in this latter sample. The single sample from a healthy animal contained rotavirus on EM examination. The results are shown in Table I.

Electron microscopic studies on the Breda "agent"

In most samples, the particles appeared rather pleomorphic (Fig. 1 (F)). We observed round particles with a diameter ranging from 60 to 200 nm, some of which presented protuberances; we also observed elongated particles ranging from 150 to 270 nm in length, some of them being narrowed in one or two places (Fig. 1 (A), (B) and (C)).

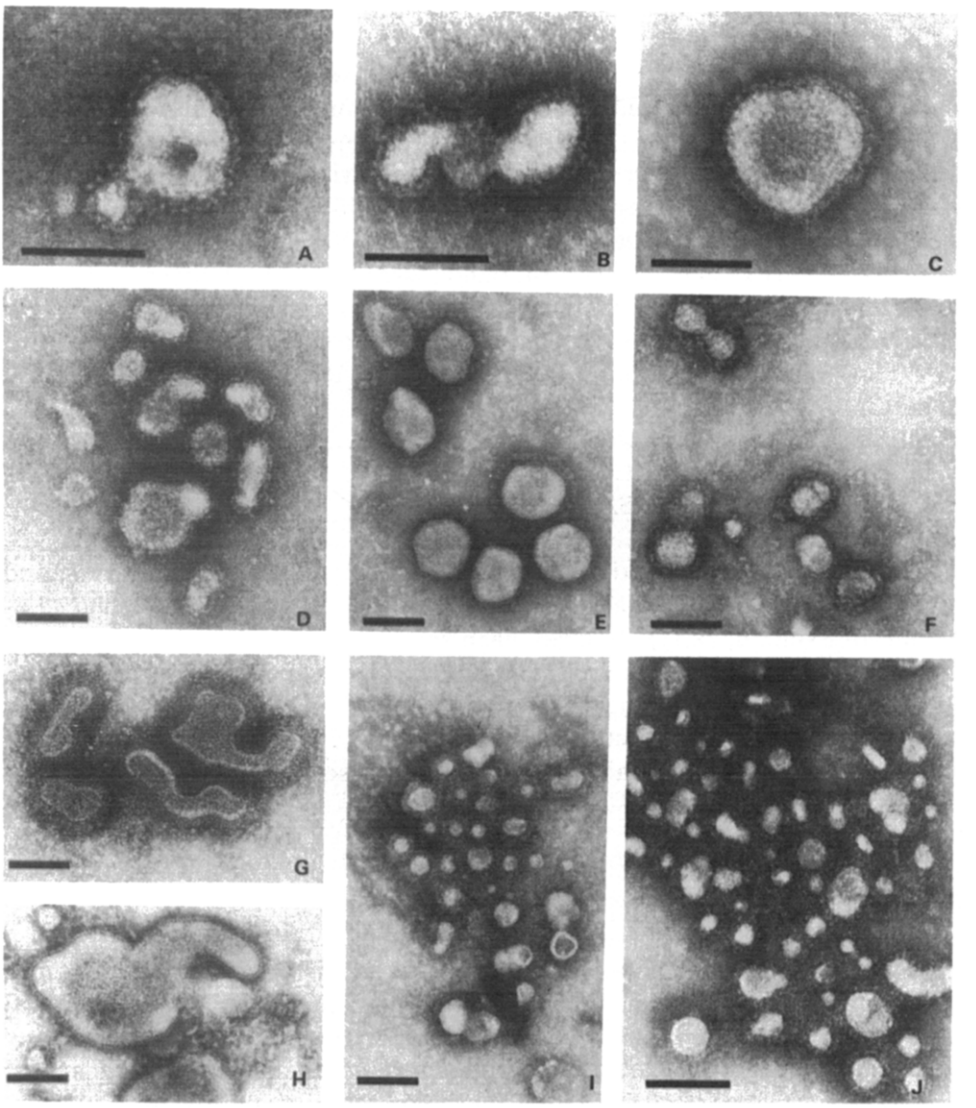

Fig. 1. (A), (B) and (C). Bovine Breda viruses. (A) Particle with a protuberance and indentation; (B) elongated-narrowed particle; (C) round particle; (D) human Breda-like particles; (E) bovine coronavirus with simple row and double row of spikes; (F) bovine Breda viruses with round and dumb-bell shaped particles; (G) bovine Breda virus coated by bovine heterologous serum; (H) artefactual particle from HRT 18 control cell supernatant; (I) bovine Breda viruses agglutinated by human convalescent serum; (J) bovine Breda viruses agglutinated by bovine homologous serum. (bar represents 100 nm.)

All particles had spikes with lengths varying from 9 to 13 nm; the 9-nm spikes were the most frequent. When compared with the spikes of BCV, these spikes seemed more flexible and more irregular, constituting a fringe rather than a corona.

Unlike BCV from cell culture, which can present particles with a simple row of short spikes and particles with a double row of short and long spikes (Fig. 1 (E)), Breda viruses observed both in feces and in cell culture supernatants did not present double rows of spikes.

In most samples, the particles were isolated (or aggregated if the fecal supernatant had been ultracentrifuged), but in two cases large spontaneous aggregates were seen.

The morphology of the bovine Breda agent is similar to that of human particles seen both in mild diarrhea and in necrotizing enteritis (Fig. 1 (D)); 736 (1) Breda particles were agglutinated by convalescent serum, but not by the acute serum of the above human patient (Fig. 1 (I)). Bovine convalescent Sera 736 (1) and 736 (2) agglutinated 736 (1) virus, and resulted in large aggregates consisting mainly of small particles (Fig. 1 (J)), whereas acute Serum 736 (1) did not agglutinate. A thick coating of antibodies was observed when the reaction was performed with a low dilution of heterologous 736 (2) serum (Fig. 1 (G)). Rabbit anti-BCV antiserum (agglutinating bovine coronavirus) neither coated nor agglutinated 736 (1) Breda particles.

With two exceptions, all cell culture supernatants from infected HRT 18 monolayers contained variable numbers of Breda particles on electron-microscopic examination. There were a few reovirus-like particles in some samples.

We noticed that there was no correlation between the number of particles seen at direct diagnosis and the number of particles seen in HRT 18 cell-culture supernatants. As inocula have been removed after viral adsorption, the particles observed in the supernatants could have been replicated by HRT 18 cells. Two samples diagnosed as Breda virus positive did not generate viral particles at a level detectable by IF or EM (Table II).

Hemagglutination and hemagglutination inhibition

In our experiments, the hemagglutinating property was not associated with all strains of Breda viruses since four samples did not produce any rat-erythrocyte hemagglutination, including the 736 (1) sample containing very numerous particles.

Hemagglutination inhibition performed on samples whose hemagglutinating titer was $\geqslant 1/16$ yielded the following results: anti-BCV serum did not significantly inhibit the hemagglutination of Breda virus-containing samples; convalescent sera from Calves 736 (1) and 92 (4) reduced the hemagglutination of only two Breda virus-positive samples out of three, and of one coronavirus-positive sample. Results are reported in Table II.

TABLE II

Presence of viruses in calf feces and cell culture: comparative results

Sample	Virological diagnosis					Growth on HRT 18 cells (first passage)					
	EM	HA	HAI			HAD on infected HRT18 cells	Immunofluorescence			Supernatant examination by EM[a]	Cytopathic effect
			736(1) Ab	92(4) Ab	BCV F15 Ab		736(1) Ab	A912 Ab	Rotavirus Ab		
736 (1)	Breda virus+++	–	NT	NT	NT	–	++	–	–	Breda virus++	CPE 1
736 (2)	Breda virus+	+1/16	–	–	–	NT	+	–	–	No virus	CPE1
736 (3)	Breda virus+	–	NT	NT	NT	–	+	–	–	No virus	Complete destruction within 24 H
92 (2)	Breda virus+	+1/32	+	+	–	–	–	–	–	No virus	CPE 1
92 (3)	Breda virus+	+1/2	NT	NT	NT	–	++	–	–	Breda virus+++	CPE 2
92 (4)	Breda virus+	+1/16	+	+	–	++	++	–	–	Breda virus++	CPE 1
197 (1)	Breda virus++	+1/4	NT	NT	NT	–	–	–	–	No virus	CPE 1
241 (1)	Breda virus (+)	–	NT	NT	NT	–	(+)	–	–	Breda virus+	CPE 1
242 (2)	Breda virus++	–	NT	NT	NT	–	++	–	–	Breda virus++	CPE 1
386 (1)	Breda virus++ Rotavirus+	+1/2	NT	NT	NT	–	+	–	–	Breda virus++ Reo like (+)	CPE 1
386 (2)	Breda virus+ Rotavirus	+1/2	NT	NT	NT	–	+	–	–	Breda virus+ Reo like (+)	CPE 1
92 (1)	Breda virus++ Rotavirus+	+1/8	–	–	–	–	++	–	–	Breda virus+++ Reo like (+)	CPE 2
461 (1)	Coronavirus (+)	+1/16	+	+	+	(+)	–	–	–	No virus	CPE 1
241 (3)	Coronavirus (+)	+1/16	–	–	+	–	–	–	–	No virus	CPE 1
419	Rotavirus+	+1/8	NT	NT	NT	–	–	–	–	No virus	CPE 1

[a] (+) <5 particles per square; + 0–5 particles per square; ++ 5–10 particles per square; +++ >10 particles per square.

Cell culture of virus

In most cases, the inoculated cell monolayers looked much the same as controls, except that the cells seemed more retracted. This aspect worsened during the following days. The cells became detached and the entire monolayer peeled off completely within 5 or 6 days, whereas control cells remained attached. We called this change CPE 1.

Two samples yielded moderately enlarged refringent cells in the whole monolayer. During the following days, the cells necrotized and detached. We called this change CPE 2.

Three samples (736 (1), 92 (2) and 92 (3)), inoculated on HRT 18 cells grown in 25-cm^2 flasks, produced a stage of cytoplasmic vacuolization. The cells then detached and holes appeared in the monolayer.

Hemadsorption carried out on the fourth day post-inoculation was negative, except for Sample 461 (1), which presented one focus of hemadsorption, and Sample 92 (4), which was clearly positive throughout the monolayer. These observations are summarized in Table II.

Immunofluorescence

When treated with rabbit hyperimmune anti-rotavirus serum, no sample showed fluorescence.

Ten samples out of 15 presented positive fluorescent reactions when treated with adsorbed convalescent serum from Calf 736 (1) (Fig. 2 (A)). The intensity of the reaction was generally correlated with the number of particles seen in the cell culture supernatants (Table II). Nevertheless, in two cases, typical Breda particles were not observed in the supernatants even when the cells demonstrated fluorescence (samples 736 (2) and 736 (3)).

Two samples containing Breda viruses (197 (1) and 92 (2)), neither developed fluorescence nor contained Breda particles in their supernatants. In addition, we found that 736 (1) Ab did not react against BCV F 15 grown on HRT 18 cells.

Buoyant density in sucrose

In the 20–45% sucrose gradients (velocity gradients) BCV F 15 and 736 (1) viruses banded nearly in the same zone: Fractions 10–14 for BCV and Fractions 11–16 for 736 (1) virus. Centrifugation to equilibrium in 20–60% sucrose gradient established that BCV F 15 virus bands as a thin ring located in Fractions 15–16, which corresponds to a density of 1.1920, whereas 736 (1) virus bands as a more diffuse ring in Fractions 15–17, which corresponds to a density of 1.1650 (Fig. 3).

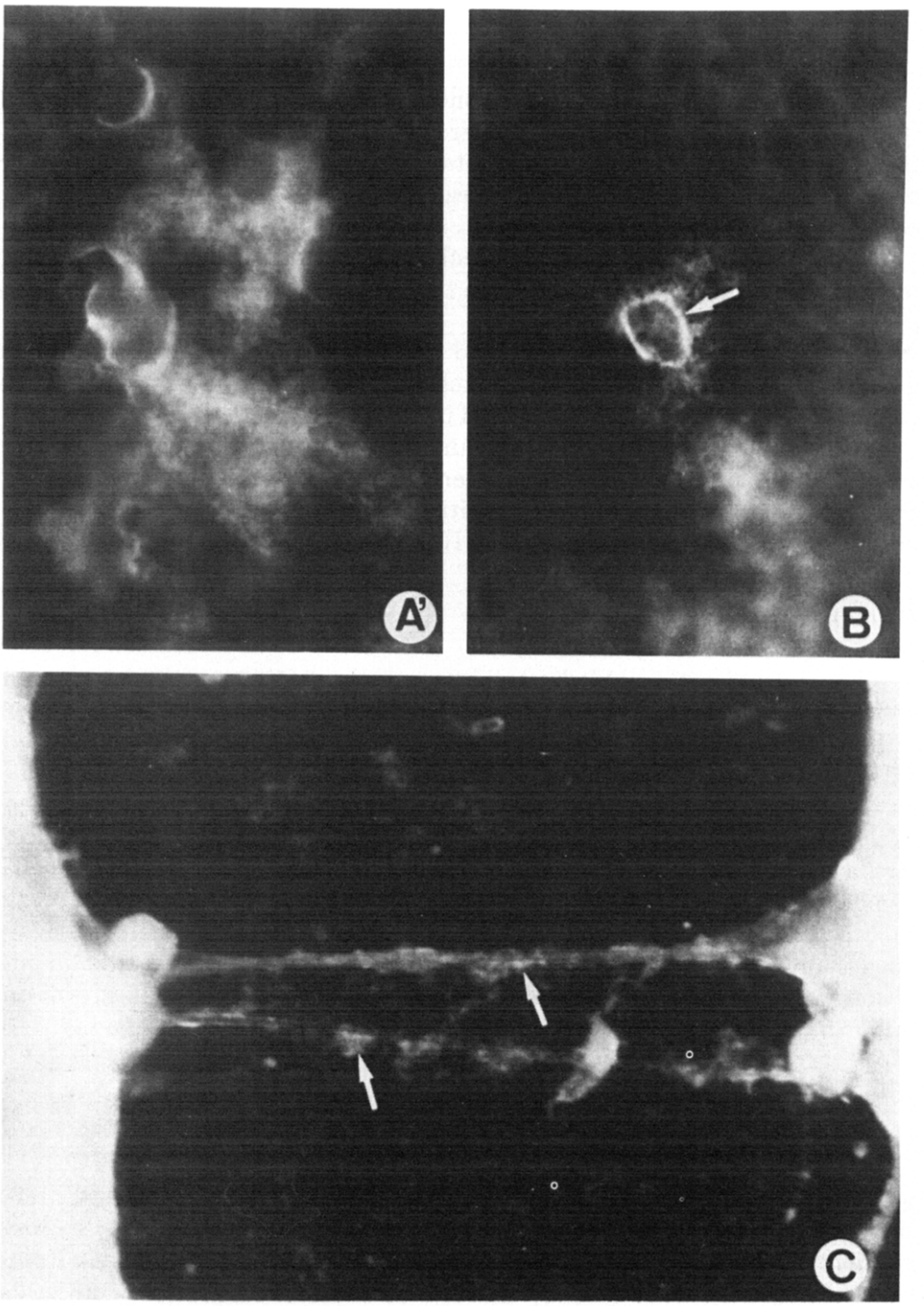

Fig. 2. (A'), (B) and (C). Immunofluorescence with Breda antisera U.S.A. on HRT 18 cells inoculated with Breda isolates. (A') Diffuse cytoplasmic fluorescence; (B) perinuclear fluorescence (arrow); (C) cytoplasmic filaments with fluorescent deposits (arrows).

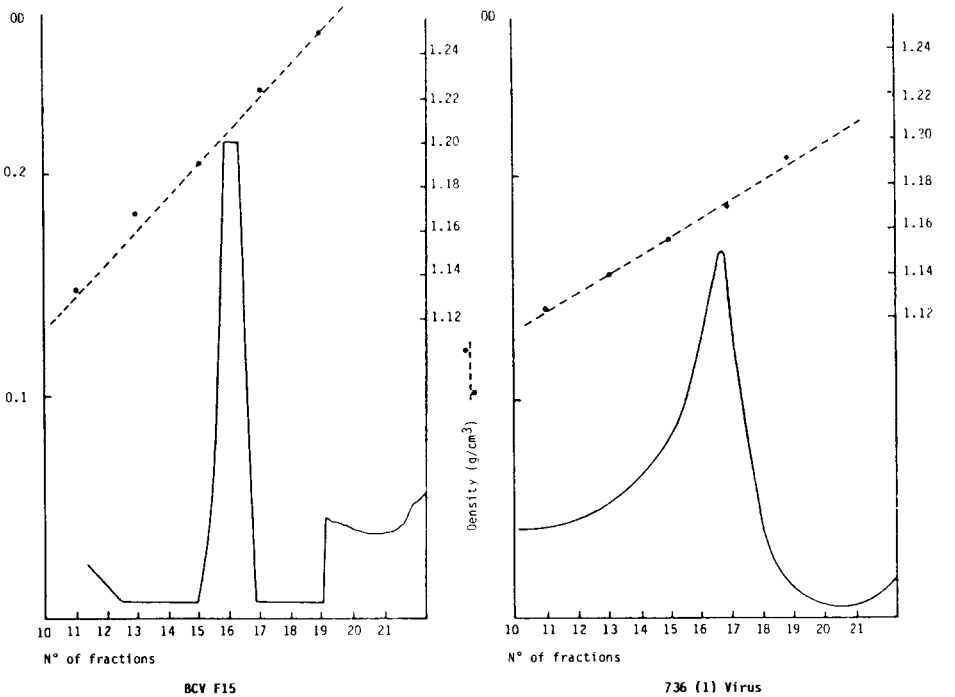

Fig. 3. Equilibrium centrifugation in 20–60% sucrose gradient.

Antigenic relationship of French isolates with U.S.A. serotypes Breda 1 and Breda 2

Ten isolates showing positive fluorescence when treated with 736 (1) antiserum were tested by IEM and IF with Breda 1 virus calf convalescent antiserum and Breda 2 virus calf hyperimmune antiserum provided by Dr. G. Woode.

All HRT 18 cell cultures inoculated with second passage supernatants from these isolates showed positive fluorescence with both antisera. Fluorescence in the cells appeared both scattered through the cytoplasm and concentrated in a perinuclear area (Fig. 2 (A') and (B)). In one case with a typical CPE, cytoplasmic filaments with thin fluorescent deposits were observed (Fig. 2 (C)).

No fluorescence was observed in control cells stained with Breda 1 and Breda 2 sera followed by rabbit anti-bovine globulin conjugated with isothiocyanate fluorescein.

Inoculated cells reacted with monoclonal bovine coronavirus antibody (A 912) did not produce any fluorescence.

IEM was performed with first-passage cell culture supernatants of the above isolates. We observed positive reactions ranging from particle coating to the

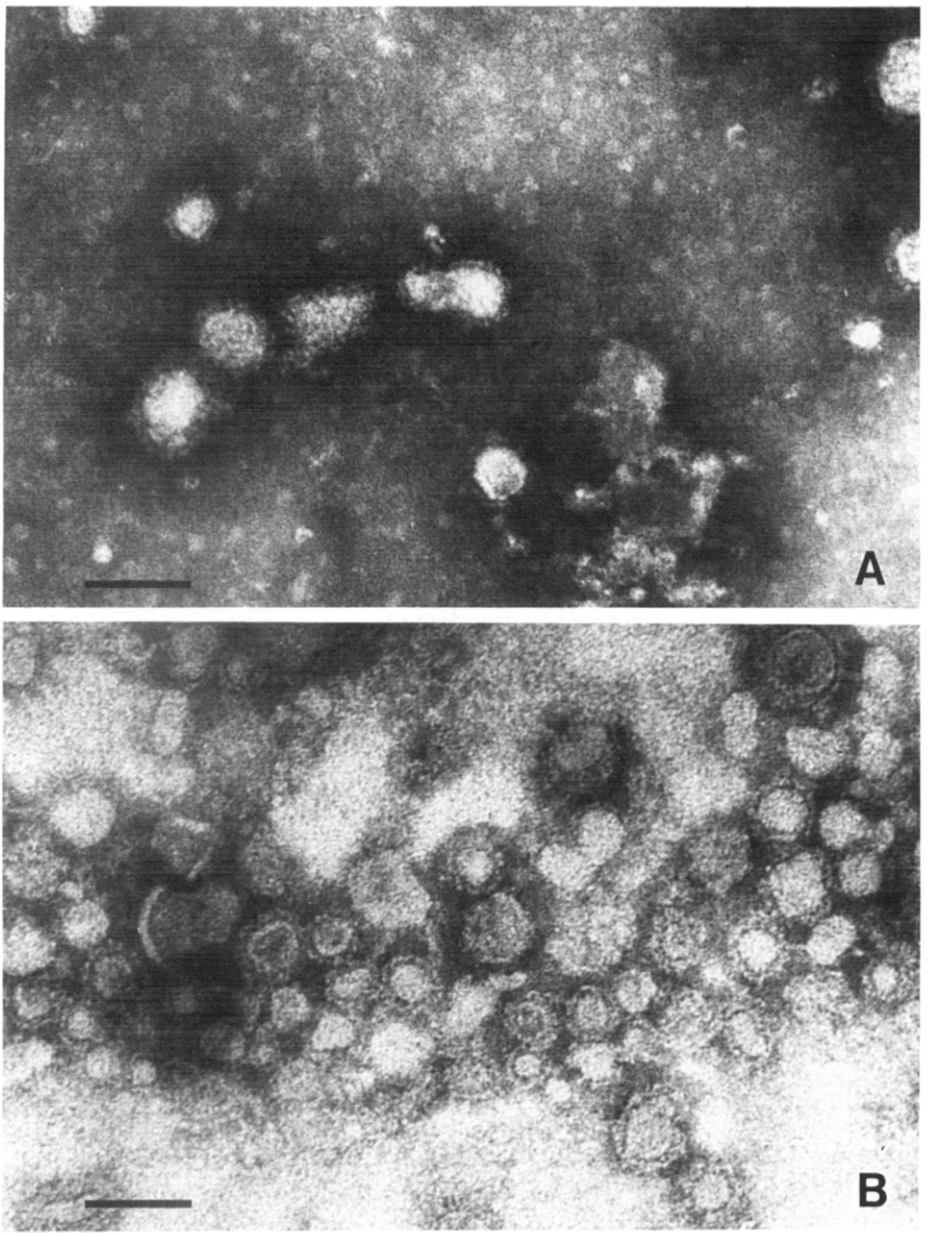

Fig. 4, (A) and (B). Immuno-microscopy with Breda antiserum U.S.A. and Breda particles from first passage on HRT 18 cells. (A) Small amount of antibody coating the particles and even uncoated particles; (B) Large aggregate obtained with Breda 2 Ab and Isolate No. 241 (2). (Bar = 100 nm.)

TABLE III

Sample	Immunofluorescence on HRT 18 cells inoculated with 2nd passage supernatants			IEM on first-passage supernatants		
	Wanda Ab (1/40)	Breda 2 Ab (1/80)	Monoclonal bovine coronavirus Ab	Wanda Ab (1/25)	Breda 2 Ab (1/100)	FCS (1/25)
736 (1)	+	–	–	+	+	–
736 (2)	+	++	–	+	+++	–
736 (3)	(+)	(+)	–	–	+	–
92 (3)	++++	++++	–	+	++	–
92 (4)	++++	++	–	++	+++	–
92 (1)	++++	++	–	++	+	–
241 (1)	+	++++	–	+	+++	–
241 (2)	+++	+	–	+++	++	–
386 (1)	+	+	–	+++	+	–
386 (2)	+	++	–	++	++	–

+++ = more than 10 particles per aggregate.
++ = 2-10 particles.
+ = coating.

constitution of very large aggregates with both antisera, Breda 1 and Breda 2. An evident qualitative difference appeared with two samples which reacted to a great extent (see Table III and Fig. 4 (A) and (B)).

DISCUSSION

Through this study, initially undertaken to establish a diagnostic method for human Breda-like virus diarrheas, some morphological, antigenic, cultural and physical data have been obtained which confirm and add to the seminal work of Woode et al. (1982).

Electron microscopy is a reliable diagnostic tool when viruses are numerous. Then sufficient particles are seen which are regular in size and whose spikes are unbroken, allowing us to conclude the presence of Breda virus. When there are fewer particles, diagnosis is more difficult since in the midst of atypical elements whose fringe is partially or totally damaged, only some particles present the typical morphological characteristics of the Breda virus; in this case only IEM can ensure that the observed particles are Breda viruses.

Generally, these viruses are of the dimensions described above, i.e. 60–200 nm for round particles, 150–270 nm for elongated ones with 9–13-nm spikes. Projections constitute a fringe which appear irregular if compared with a human paramyxovirus previously studied (Fleury et al., 1983). Variations observed in peplomer length could be explained by the presence of a double row of spikes as we already observed in bovine coronavirus, though we have never seen a double row of spikes in Breda virus-positive samples.

Larger particles, 200–500 nm, have been occasionally seen both in fecal samples and cell-culture supernatants. The viral nature of such particles is uncertain, owing to the fact that such large particles are not encountered in immunoaggregates which are composed, mainly, of 60–100-nm particles. The large particles might, in fact, be fragments of intestinal epithelial cells. Nevertheless, as enveloped viruses can be very pleomorphic, the possibility that large particles are viruses cannot be ruled out. Small atypical particles with less defined spikes or no spikes probably represent either damaged or incomplete particles. We observed such particles either after purification of Breda viruses and bovine coronaviruses or in Breda-positive cell-culture supernatants.

Bovine Breda viruses are related to human Breda-like particles, but not to bovine enteric coronavirus as shown by IEM; some isolates reacted to a great extent with Breda 1 or Breda 2 sera from the U.S.A. and probably represent members of these serotypes.

Preliminary results of in vitro culture associating cellular degenerative process, positive IF and presence of Breda particles in the supernatants allow us to assume that certain strains could have replicated. Samples which contained Breda and rotaviruses yielded Breda viruses and reo-like viruses (without S particles) detectable only by EM. Subsequent culture will show if this double replication continues.

So far, the three Breda virus isolates previously described (Woode et al., 1985) possess hemagglutinin. In our study, only 3 out of 12 Breda isolates (25%) demonstrated a significant hemagglutinating titer, and only one produced hemadsorption on HRT 18 cells. This could signify that not all Breda viruses are hemagglutinating, or that hemagglutinating spikes could be partially coated by intestinal antibodies which could prevent agglutination of rat erythrocytes.

Woode et al. reported difficulties in detecting Breda virus antibodies by the HI test. This suggests that either all Breda viruses do not possess hemagglutinating activity or anti-hemagglutinin antibodies do not appear in all animals infected by the Breda virus. Furthermore, Woode described a strain (Ohio strain) which had little or no HA titer.

In conclusion, it must be pointed out that Breda viruses have been found in Europe as well as in the U.S.A. and are probably widespread throughout the world, as are rotaviruses. In our study, they were associated with calf gastroenteritis in 60% of the cases examined. Severe symptoms such as hemorrhagic enteritis and dehydration were observed, especially when animals were submitted to inadequate conditions, such as battery rearing. This high rate of infection is consistent with the observation that 88.5% of cattle have Breda virus antibodies (Woode et al., 1985).

Bovine Breda particles have been antigenically related to human particles by IEM (Beards et al., 1984), and our electron microscopic studies of bovine and human particles suggest that they represent members of the same family;

Woode et al. (1985) on the basis of morphological and antigenic properties have suggested the relationship of Breda viruses to the equine Berne virus; the buoyant density in sucrose of 1.16 we found from one Breda isolate is comparable to that of Berne virus, but further investigations using Berne antiserum would be needed to confirm this hypothesis.

The ability to culture in vitro some strains of Breda virus will permit future development of virological studies on the Berne/Breda virus group, including comparative studies with the human Breda viruses.

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