

# Human-Enteric-Coronaviruslike Particles (CVLP) With Different Epidemiological Characteristics

Marc Sitbon

*Centre International de Recherches Médicales de Franceville, Franceville, Gabon*

One hundred fifty-six diarrheic and 115 control stools collected throughout a year from nonhospitalized children were examined by electron microscopy in Haut-Ogooué, Gabon; 65.2% of the controls and 38.5% of the diarrheics were found to contain coronaviruslike particles (CVLP). In both diarrheic and control groups the CVLP prevalences showed a seasonal variation whereas significant variation of prevalence with age was observed only in the controls. Thus, the CVLP in controls were significantly more abundant in children over 2 years old (76% vs 48%,  $P < .01$ ) and more frequently observed during the months of rainy seasons (75% vs 54%,  $P < .02$ ). On the other hand, the higher prevalence of CVLP in diarrheics over 2 years old was not significant (48% vs 36%,  $P = .20$ ), whereas a significantly lower prevalence of CVLP during the months of rainy seasons was observed in this group (27% vs 50%,  $P < .01$ ). Studies of the climatological factors in this equatorial climate showed a parallel cyclical variation of parameters representing rainfall, temperature, as well as relative humidity. We were not able to distinguish which of these factors was influencing more directly the prevalence of CVLP.

**Key words:** diarrheic, coronaviruses, human, seasonal prevalence, Gabon

## INTRODUCTION

The role of coronaviruses in gastroenteritis has been extensively studied in animals, from which several strains causing diarrheic have been isolated from different species [Garwes, 1982]. Since the first electron microscopic (EM) examinations of human enteric particles resembling coronaviruses in India [Mathan et al, 1975] and in England [Caul et al, 1975], coronaviruslike particles (CVLP) [Macnaughton and Davies, 1981] have been suspected to cause several sorts of outbreaks of gastroenteritis in humans [Chany et al, 1982; Vaucher et al, 1982]. However, conflicting data

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Dr. Sitbon's present address is Laboratory of Persistent Viral Diseases, Rocky Mountain Laboratories, Hamilton MT 59840.

Address reprint requests to Dr. Marc Sitbon, Rocky Mountain Laboratories, Hamilton MT 59840.

have been reported concerning their role in this disease [Caul and Egglestone, 1982], and their viral nature has also been questioned [Dourmashkin et al, 1980]. Furthermore, from epidemiological surveys performed in different geographical areas by EM observation of fecal extracts, it seems that human enteric CVLP were observed more frequently in tropical areas than in Europe or North America [Macnaughton and Davies, 1981]. The reasons for these differences are not known. Because there is no serological test for them, CVLP can only be identified by EM according to morphological characteristics previously established from virus isolates [Caul et al, 1977; Macnaughton and Davies, 1981]. In this article, we report a very high prevalence of CVLP in stools of nonhospitalized control and diarrheic children of an equatorial African country, Gabon. From the differences observed in the prevalence of CVLP in both control and diarrheic children according to the seasonal and age group studied, we here postulate the existence of at least two biologically different varieties of CVLP in human feces and consider the possible influence of relative humidity (RH) as well as temperature and rainfall in their prevalence.

## MATERIALS AND METHODS

### Geographical Situation and Study Population

This study was carried out in the southeastern part of the Gabonese Republic (Africa) in the province of Haut-Ogooué (Fig. 1). This study included urban and rural populations living in a zone of equatorial forest and plateau savanna (Fig. 1). Diarrheic stools were collected between December 1980 and December 1981 from 156 nonhospitalized diarrheic children under 10 years of age. Collecting in rural areas was performed with the help of the Service des Grandes Endémies in the villages of

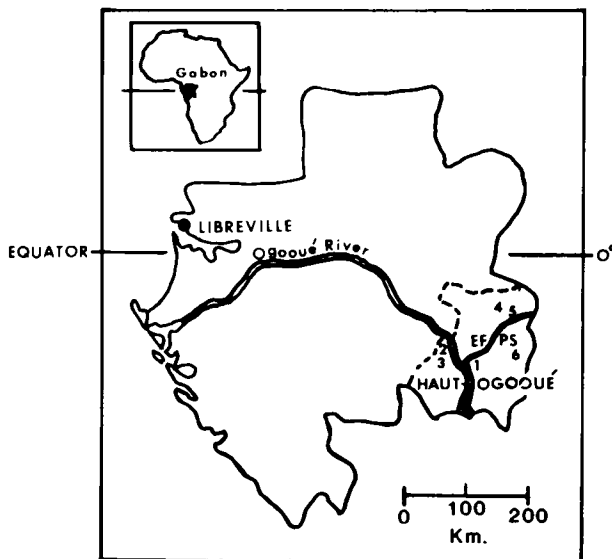


Fig. 1. Map of Gabon showing the province of Haut-Ogooué and the different places of stool collecting. 1, Franceville; 2, Mounana; 3, Moanda; 4, Ambinda; 5, Bouala-Youma; 6, Saye. The delimitation between the equatorial forest (EF) and plateau savanna (PS) areas is indicated by the solid line running from northeast to south of the province.

Ambinda, Bouala-Youma, and Saye (Fig. 1). In urban areas, stools were obtained through the daily medical consultations of (1) the hospital of the Compagnie des Mines d'Uranium de Franceville (COMUF) situated in the town of Mounana, (2) the hospital of the Compagnie des Mines de l'Ogooué (COMILOG) situated in the town of Moanda, and (3) the medical center of the Caisse Nationale de Sécurité Sociale in Franceville (Fig. 1). Children were considered as diarrheic when they defecated three or more soft-to-liquid stools in 24 hours. One hundred fifteen (115) nonhospitalized control children under 10 years of age were documented to have neither gastroenteritic nor respiratory clinical signs. Their stools were collected in both rural and urban areas from March to December 1981.

### **Electron Microscopy (EM)**

Fresh fecal samples were collected in sterile vials and brought to the Centre International de Recherches Medicales, de Franceville (CIRMF). Samples were processed or frozen at  $-20^{\circ}\text{C}$  at the CIRMF within 48 hours after collection. Five to 30 gm of feces was thoroughly homogenized in sterile double-distilled water (about 20% w/v) and then clarified at 4,000g for 20 minutes. The supernatants were separated from the pellets and stored at  $-70^{\circ}\text{C}$ . One drop of fresh or thawed supernatant was deposited on a strip of parafilm. Two carbon-collodion-coated copper grids were applied to each sample and then touched with a filter paper to blot the excess fluids. The grids were then dipped in a drop of distilled water before being floated on a drop of 4% phosphotungstic acid (PTA) at pH 6.5 and then blotted again with filter paper. Observations were made using a Elmiskop 101 (Siemens) electron microscope at 80 kV in Franceville (Gabon) and a Philips 301 at 60 kV in Paris (France). Magnifications were calibrated using 85-nm latex spheres (E. Fullam, Inc., Schenectady, NY).

### **Meteorological Data**

Meteorological data were kindly provided by the Service de la Météorologie Nationale (Libreville, Gabon) from daily surveys carried out at the International Airport of M'Vengué and by the Station Agronomique d'Okoloville, respectively located at about 40 km east and north of Franceville, Haut-Ogooué (Fig. 1). The data included the daily maximum and minimum temperatures and relative humidity, as well as monthly rainfalls.

### **Statistical Analysis**

For statistical comparisons, we used the chi-square test ( $\chi^2$ ) with 1 degree of freedom. A difference was considered significant when  $\chi^2$  was greater than 3.84 ( $P < .05$ ).

## **RESULTS**

### **Morphology of the Coronaviruslike Particles (CVLP)**

CVLP were very pleomorphic and generally ranged from 80 to 300 nm in greatest dimension (Fig. 2A). Nevertheless, round forms (Fig. 2B), or forms over 600 nm in diameter were often observed (Fig. 2C). Particles were considered as CVLP because of the presence of a typical arrangement of regular projections ranging from 15 to 45 nm in length [Caul et al, 1977; Macnaughton and Davies, 1981] (Fig.

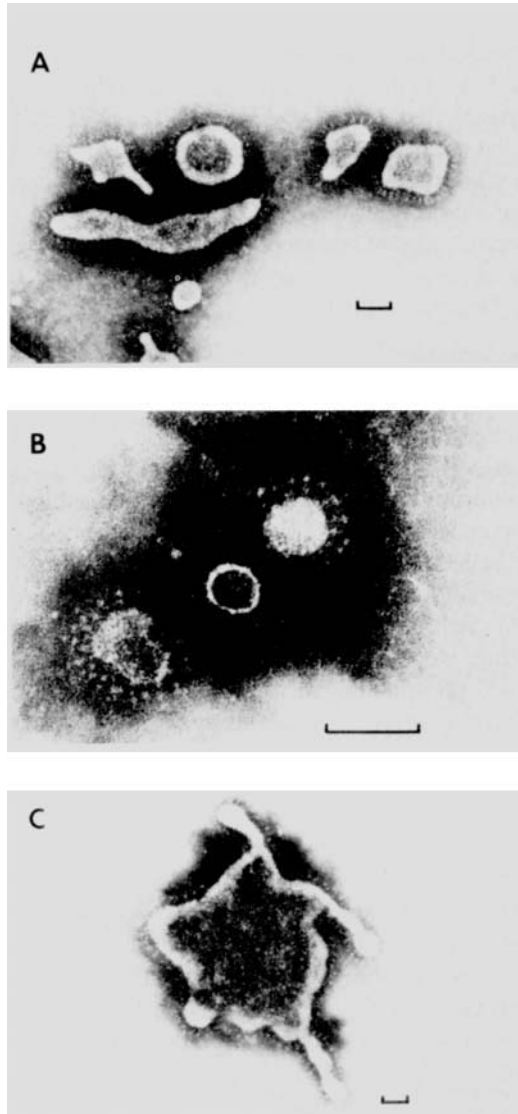


Fig. 2. Electron micrographs of negatively stained coronaviruslike particles (CVLP) from childrens' stools showing (A) pleomorphism of the CVLP in the same stool including single- and double-fringed particles, (B) round CVLP, and (C) irregular-form, over 600 nm in size. In all cases projections were between 15 and 45 nm with an average of approximately 20 nm. Bar = 100 nm.

2). CVLP were easily distinguishable from previously described cellular vesicles [Maestracci, 1976; Caul et al, 1977]. These cellular vesicles, frequently observed in the stools, presented shorter projections arranged in an ill-defined crown and were not scored as CVLP. No differences were seen between the CVLP observed in control and diarrheic stools. For this reason, and since a wide variety of CVLP forms was observed in every positive stool with the same range of concentrations in both control and diarrheic groups, we scored the stools as positive or negative for the presence of

CVLP without any regard to the concentration and the types of CVLP we observed in the sample.

### Prevalence of CVLP in Control and Diarrheic Children

One hundred fifteen (115) nondiarrheic stools collected from different control children were examined by EM. Of these, 75 (65.2%) contained CVLP at various concentrations. We also examined 156 diarrheic stools and found that 60 (38.5%) contained CVLP. Since the difference between the prevalence of the CVLP in these two populations was statistically highly significant ( $\chi^2 = 18.9$ ;  $P < .001$ ), we examined the prevalence of CVLP as a function of age and season.

### Prevalence of the CVLP in Different Age Groups and Comparison of Control and Diarrheic Children

We compared the prevalence of CVLP between children up to 2 years old (group I) and children from 2 years to 10 years old (group II) (Table I). Among the controls, CVLP were significantly less frequent in the group I (47.7%) compared to the group II (76.1%) ( $\chi^2 = 9.6$ ,  $P < .01$ ). However, no statistically significant difference was observed between the two age groups in the diarrheic stools (36.4% versus 42.8%,  $\chi^2 = 0.5$ ,  $P > .30$ ). Moreover, we found that the CVLP prevalence in control stools was significantly higher than in diarrheic stools only among the children of the group II (76.1% and 42.8%, respectively;  $\chi^2 = 13.6$ ,  $P < .001$ ) (Table I). The difference observed in group I between control and diarrheic stools was not statistically significant (respectively 47.7% versus 36.4%;  $\chi^2 = 1.6$ ,  $P = .20$ ). Thus, higher prevalence of CVLP in control subjects of group II alone accounted for the difference in prevalence between control and diarrheic children.

### Seasonal Prevalence of the CVLP and Comparison in Control and Diarrheic Children: Relation to Climatological Factors

The monthly prevalences of CVLP in nondiarrheic and diarrheic stools shown in Figure 3 indicated that the prevalences observed in the two populations fluctuated in opposite directions. Nevertheless, we observed that the amplitudes of variation were higher in the group of diarrheic children as compared with controls. We wanted to compare these fluctuations with changes in climate. In the area of our study, there is a short dry season from December to January, a long rainy season from February to May, a long dry season from June to September, and a short rainy season from October to November. Previous studies in areas with similar climates generally

TABLE I. Incidence of the CVLP According to the Age Group in Control and Diarrheic Children Under 10 Years of Age

Age group	Control stools		Diarrheic stools		$\chi^2$ <sup>a</sup>
	With CVLP/ total	(%)	With CVLP/ total	(%)	
≤2 years (I)	21/44	(47.7)	39/107	(36.4)	1.6; $P = 0.20$
2-10 years (II)	54/71	(76.1)	21/49	(42.8)	13.6; $P < 0.001$
$\chi^2$ <sup>a</sup>	9.6; $P < 0.01$		0.5; $P > 0.30$		

<sup>a</sup>Statistical significance of the different incidences was tested using the chi-square test with 1 degree of freedom within the same age group and within the control and diarrheic group.

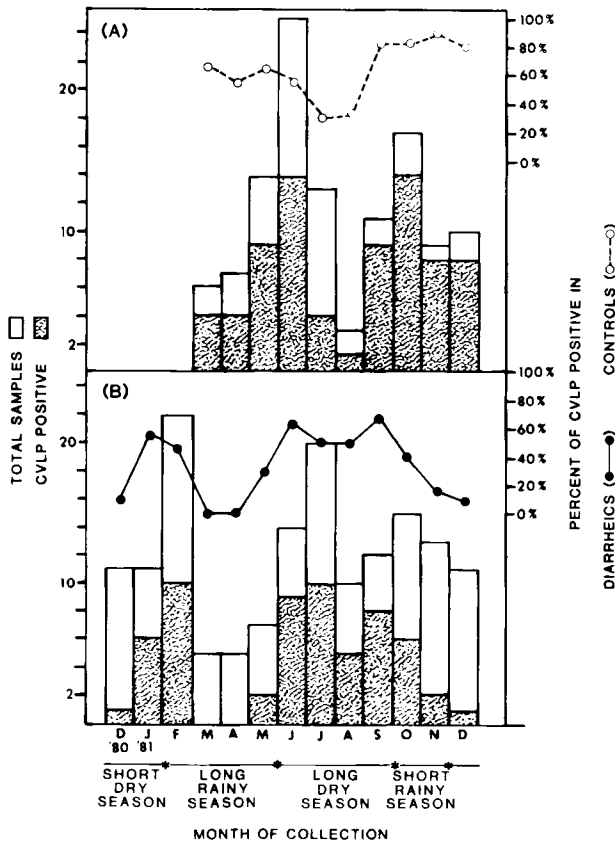


Fig. 3. Monthly prevalence of CVLP in nonhospitalized children under 10 years of age. Stools from (A) control children were collected from March to December 1981, and from (B) diarrheic children were collected from December 1980 to December 1981.

recorded the monthly rainfall and the monthly average of the daily maximum and minimum levels of temperature and relative humidity (RH) [Hieber et al, 1978; Viera de Torres et al, 1978; Soenarto et al, 1981; Paniker et al, 1982]. In their cases, although rainfall usually varied with the season, temperature, and, for the most part, RH, showed low fluctuations. In the area of our study, we found that fluctuations of monthly rainfall and monthly average of daily maximum temperature followed the classical seasonal division (Fig. 4A,B). As expected, the monthly average of daily maximum and minimum RH did not show any obvious seasonal fluctuations (not shown). However, we found that the monthly average of the calculated daily difference between these two parameters ( $\Delta$ RH) showed a clear seasonal fluctuation which followed a cycle parallel to the classical division of seasons (Fig. 4C).  $\Delta$ RH represented the monthly average of the amplitude of the daily RH variations.

Although the CVLP prevalence in control stools was high in every month of our study, we observed a lower prevalence during the months of dry seasons (Fig. 3A), whereas the CVLP prevalence in the diarrheic stools was highest during the dry months (Fig. 3B). For comparison, we pooled the data in two groups including the samples collected during either dry or rainy months. Since the rainfall was unusually high in December 1981 (Fig. 4A), the data from this month were included in the

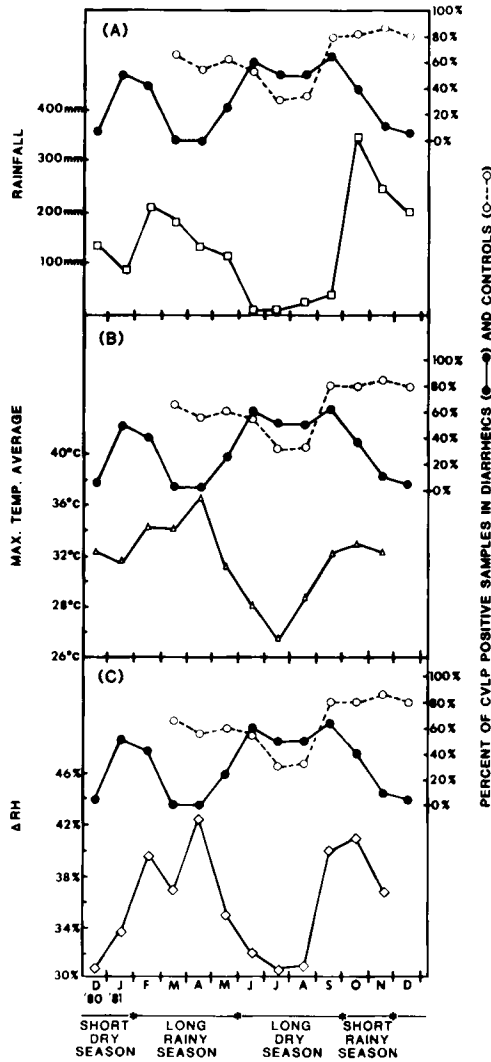


Fig. 4. Variations of climatological parameters and prevalence of CVLP in control and diarrheic children. Comparison of the monthly prevalences of CVLP with (A) □, monthly rainfall; (B) △, monthly average of the daily maximum temperatures; and (C) ◇ monthly average of the daily variations of relative humidity (ΔRH).

group of rainy season months (Table II). Table II showed a higher prevalence of CVLP in control stools during the rainy months (74.6%) as compared to the dry months (53.9%) ( $\chi^2 = 5.4, P = .02$ ) (Table II). On the other hand, we found a highly significant lower prevalence of CVLP in diarrheic stools during the rainy months (26.9%) as compared to the dry months (50%) ( $\chi^2 = 8.7, P < .01$ ) (Table II).

**DISCUSSION**

We found coronaviruslike particles (CVLP) very frequently in stools from healthy and diarrheic children living in Haut-Ogooué. At the same time, we also

**TABLE II. Incidence of the CVLP According to the Seasonal Month Group in Control and Diarrheic Children Under 10 Years of Age**

Seasonal group	Control stools		Diarrheic stools		$\chi^2$ <sup>a</sup>
	With CVLP/ total	(%)	With CVLP/ total	(%)	
Rainy months <sup>b</sup>	47/63	(74.6)	21/78	(26.9)	31.7; P < .001
Dry months <sup>c</sup>	28/52	(53.9)	39/78	(50)	0.1; P > .50
$\chi^2$	5.4; P = 0.02		8.7; P < .01		

<sup>a</sup>Statistical analysis was performed as mentioned in Table I.

<sup>b</sup>Rainy months included February, March, April, May, October, November, and December 1981.

<sup>c</sup>Dry months included December 1980, January, June, July, August, and September 1981.

observed a high prevalence of CVLP in healthy and diarrheic adults living in the same area (data not shown). CVLP were found in diverse groups of individuals with different dietary habits. These included individuals from the indigenous ethnic groups as well as other Africans, Europeans, and North Americans living in Gabon (data not shown). This observation and the fact that we found CVLP frequently in domestic animals (goats and dogs) and in primates maintained in captivity at the CIRMF (data not shown) suggested that the higher prevalence of CVLP in tropical countries [Macnaughton and Davies, 1981] is not due to the different alimentary habits or to metabolic differences [Mathan et al, 1975] but rather to the higher prevalence of a transmissible agent.

Interestingly, we found that CVLP were more frequently present in control children than in diarrheic children, as did Schnagl et al in studies on Australian aboriginals [1978]. Moreover, CVLP in the controls were more abundant in stools of children over 2 years old than in children under 2 years old, whereas no significant age difference was found in the diarrheic group. However, in children over 2 years of age (group II), a significantly higher prevalence of CVLP in controls than in diarrheic patients was observed (Table I). The fact that this was not observed in the group I subjects indicated that the reduced CVLP prevalence in diarrheics might be primarily a characteristic of the age group and secondarily an effect of the diarrhea. The reason(s) for this age-dependent difference remains to be elucidated, but it could be explained by the existence of different CVLP in the two age groups. The different seasonal variations of CVLP in both control and diarrheic stools seemed to indicate the existence of two or more groups of CVLP with different epidemiological characteristics.

We did not observe any obvious morphological difference between CVLP present in control and diarrheic stools. However, recent findings obtained from cats bred in a barrier-maintained colony showed the existence of infectious enteric CVLP morphologically and serologically distinguishable from the formerly described feline enteric coronaviruses [Stoddart et al, 1984].

An alternative explanation for the apparent epidemiological difference in CVLP of controls and diarrheics is that the decreased prevalence of CVLP in diarrheics could have been a consequence of the presence of another agent which possibly interfered with the CVLP infection. This hypothesis seemed unlikely since the only infectious agent for which we also found seasonal variation was rotavirus, which also peaked during the dry months [Sitbon et al, 1985] and which was found frequently in association with CVLP (data not shown).



We observed a significant correlation between the variation in prevalence of CVLP and the seasonal cycle. However, we did not observe an exact relation between the CVLP prevalences and the level of any of the three climatological parameters studied (Fig. 4). Thus, the highest or lowest prevalences of CVLP in both controls and diarrheics were not observed during the months presenting the highest or lowest levels of these parameters (Fig. 4). It is possible that the cyclical variation in prevalence of CVLP depends on a complex interaction between these three climatological parameters. Alternatively, there may exist other unknown cyclical environmental factors which play a role in this apparent seasonal variation. Comparative studies of the prevalence of CVLP in tropical and temperate settings in relation to all the climatological factors might help in understanding the strikingly different prevalences of CVLP observed in these geographical areas. The existence of biologically distinct, but morphologically indistinguishable, CVLP might account for the conflicting reports on the role of the coronaviruses in gastrointestinal diseases.

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