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Shedding of Coronavirus-Like Particles by Children in Lesotho

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Stools from 266 children in four districts of Lesotho were examined for viruses by electron microscopy (EM) over a 5-week period. Eighty one (30.5%) of the children shed coronavirus-like particles (CVLPs). Shedding was not significantly associated with diarrhea. The proportion of children shedding these particles increased with increasing age. In one district, the prevalence of CVLP shedding (67.9%) was at least twice the prevalence in any of the other three districts. This was the only district to be sampled during the first week of the study. It was shown that the proportion of children shedding CVLPs declined during each of the 5 weeks of study. The stools of six children who shed CVLPs in the early summer of 1984–85 were negative by EM 5 months later. There was no association between the shedding of pathogenic bacteria or parasites and the presence or absence of CVLPs in the stool.

KEY WORDS: coronavirus, enteric infection, Africa

INTRODUCTION

Several animal species are known to be susceptible to enteric infection by coronaviruses [Garwes, 1982], usually resulting in severe diarrhea. Particles resembling coronaviruses in human stool have been described by several workers, notably in India [Mathan et al., 1975], Australia [Schnagl et al., 1978, 1979], and Gabon in West Africa [Sitbon et al., 1985]. It has been difficult to attribute a causative role to these particles in diarrhea, as healthy individuals can also shed them [for a review, see MacNaughton and Davies, 1981]. Nevertheless, there have been several reports of gastroenteritis in humans apparently caused by coronavirus-like particles (CVLPs) [Caul et al., 1975; Gerna et al., 1985; Rettig and Altshuler, 1985; Vaucher et al., 1982]. There is still controversy over whether these particles are truly viruses [Dourmashkin et al., 1980], although there have been reports of successful propagation in organ cultures [Caul and Egglestone, 1977; Resta et al., 1985], of antigenic differences [Schnagl et al., 1986], and of preliminary biochemical characterization [Resta et al., 1985; Battaglia et al., 1987]. Schnagl et al. [1987] recently reported widely different electrophoretic profiles for the proteins of CVLPs from different sources, which sheds doubts on their homogeneity as a group. CVLPs have recently been described in the stools of individuals with AIDS or AIDS related complex (ARC) [Kern et al., 1985] and homosexual men whose HIV antibody status was unknown [Riordan et al., 1986].

The data described in this paper were collected as part of a health impact evaluation of a rural water supply project [Esrey et al., 1987]. No difference in the shedding of CVLPs was found between children from villages with an improved water supply compared with children from villages which relied on traditional contaminated water sources [Esrey et al., 1987]. The present paper reports on the electron microscopic appearance of the CVLPs found and the prevalence of shedding in relation to factors commonly associated with infection by enteric pathogens in general. Apart from the study of Sitbon in the Republic of Gabon, West Central Africa [Sitbon, 1985], there have been no other large-scale studies on the shedding of coronavirus-like particles in Africa.

MATERIALS AND METHODS

Geographical Situation and Study Population

Data used in this paper were collected from 21 rural villages in the Kingdom of Lesotho, which is landlocked by South Africa. All villages were situated in the lowlands or foothills (Fig. 1), in which the majority of the population resides, and were selected from four of the ten administrative districts. Villages ranged in elevation from 1,400 metres to 2,000 m. Within each village households were selected randomly and all chil-

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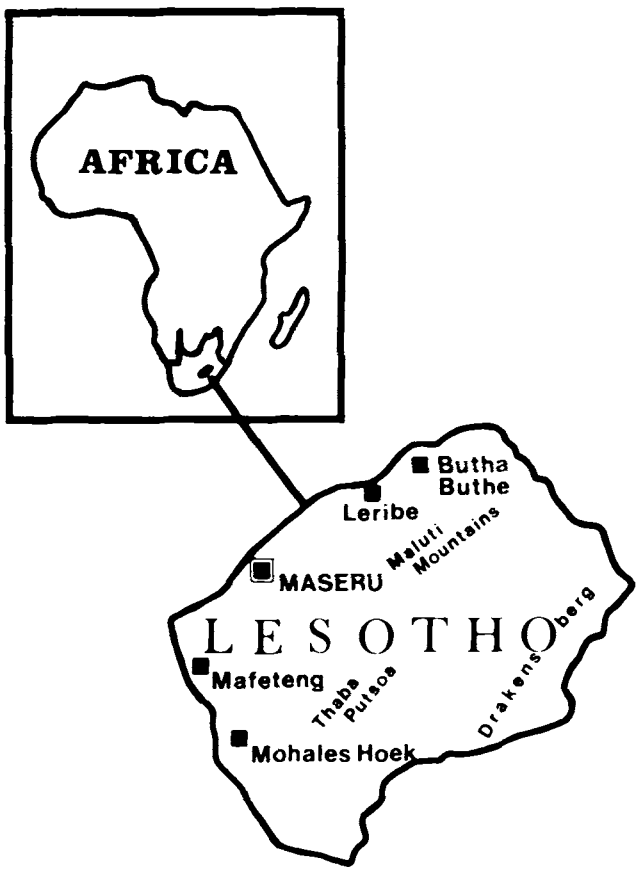

Fig. 1. Map of Lesotho showing the main towns and villages after which the administrative districts are named.

dren under 5 years of age within the household were enumerated. Starting in July–August 1984, and continuing for approximately 6 months, an average of 28 children were studied in each village. Stool samples were taken from approximately 45% of these children (n = 266) over a 5-week period in October–November 1984, at the start of the wet summer season.

Examination of Stool Specimens

Stools were collected by mothers and placed in 60-ml plastic containers (Sterilin). The specimens were packed in ice within 4 hours, were flown to Johannesburg, and arrived on the same day as collection. After samples were taken by the bacteriology laboratory of the South African Institute for Medical Research, the specimens were kept at 4°C and forwarded to the Virology Institute usually on the following day.

Stools were examined for viruses by electron microscopy (EM) using standard procedures. Briefly, stools were prepared as 10–20% suspensions in distilled water and clarified at 3,000 rpm for 30 minutes. A drop of the supernate was applied to a carbon-Formvar-coated EM grid and the excess fluid was removed with torn filter paper. Potassium phosphotungstate (2%) at pH 7 was applied to the grid and the excess removed as before. The grid was examined in a Jeol 1200 EX electron microscope. All specimens which were negative for CVLPs by this method were re-examined after ultracentrifugation of the clarified stool suspension for 1 hour at 37,000 rpm and resuspension of the pellet in 1–2 drops of 0.1% bacitracin. These preparations were applied to grids and stained as above. Standard bacteriological procedures were used to isolate *Campylobacter* spp., *Escherichia coli*, *Salmonella* spp., *Shigella* spp. and *Yersinia* spp. [Esrey et al., 1987]. Laboratory personnel were blind as to the age and sex of the child, village type and location, and other study variables. Data were analysed using SYSTAT [Wilkinson, 1987] on a personal computer.

RESULTS

Viruses Shed

Coronavirus-like particles were found in 81 of the 266 stool specimens (30.5%). Astrovirus was found in one specimen, and adenovirus in six others, one of which also contained CVLPs. Since CVLPs were the only virus-like agents detected with any frequency, only results related to CVLPs are given below.

Morphology of Coronavirus-Like Particles

The CVLPs were pleomorphic, ranging from 80 to 740 nm in their greatest dimension. They had regular projections and were similar morphologically to the CVLPs described by other authors [Mathan et al., 1975; Caul and Egglestone, 1977; Riordan et al., 1986; Schnagl et al., 1987]. Not all of the fringes (coronas) of CVLPs in specimens from different children were exactly alike (Fig. 2), and in two cases particles with distinctly dissimilar fringes were seen in the same specimen.

Prevalence of Coronavirus-Like Particles

The proportion of children shedding CVLPs increased with increasing age, from 21.3% among children less than 13 months of age to 44.4% among children aged between 37 and 48 months (Table I). However, the difference in the proportion of children of different ages shedding CVLPs was of doubtful significance ($P > 0.05$). Among 4-year-old children, the proportion of positives dropped to 19.0%. There was no bias of age groups in the different districts ($P > 0.5$). More males (35.5%) than females (26.2%) shed CVLPs, but this was not significant ($P > 0.1$).

According to the mother's definition of diarrhea, 9.4% of the children were reported to have diarrhea during the 24-hour period prior to stool collection. No significant association between the diarrhea status of children and the presence or absence of CVLPs in the stool was found ($P > 0.9$; Table II).

In three of the districts shown in Figure 1, the proportion of children shedding CVLPs did not exceed 25% (Table III). In a fourth district, Butha Buthe, the prevalence of CVLP shedding was at least twice the prev-

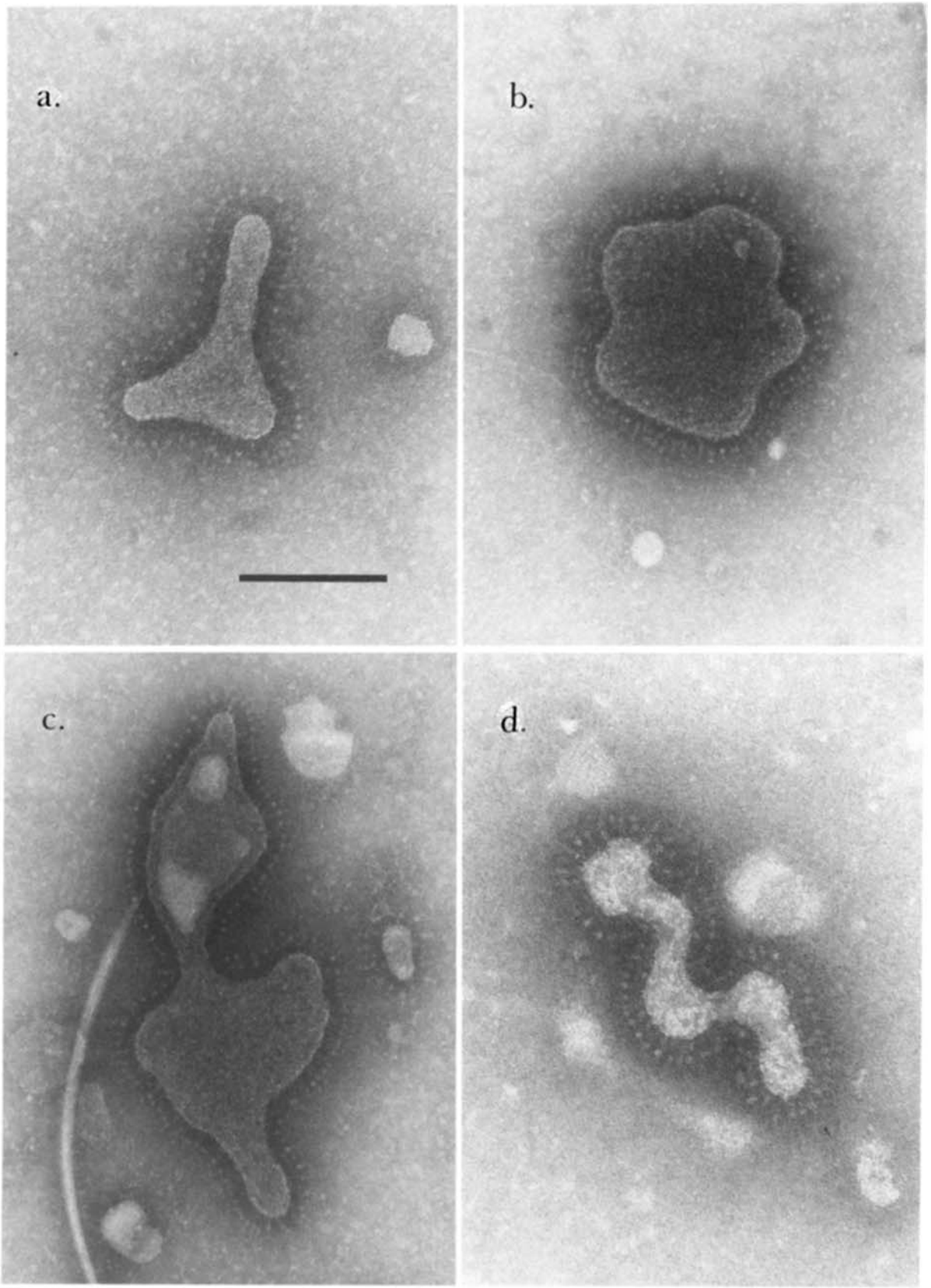

Fig. 2. Electron micrographs of coronavirus-like particles found in the stools of children from Lesotho. Panels **a** and **b** show different particles from the same child. Panels **c** and **d** show particles from different children. Bar represents 200 nm.

TABLE I. Prevalence of CVLP Shedding by Age at the Time of Stool Collection

Age (mo)	Sample size	% (no.) positive for CVLPs
13	47	21.3 (10)
13–24	69	30.4 (21)
25–36	63	34.9 (22)
37–48	45	44.4 (20)
48	42	19.0 (8)
Total	266	30.5 (81)

TABLE II. Prevalence of CVLP Shedding by Diarrhea Status During the 24-Hour Period Prior to Stool Collection

Diarrhea	Sample size	% (no.) positive for CVLPs
No	232	30.2 (70)
Yes	25	28.0 (7)
Unknown	9	44.4 (4)
Total	266	30.5 (81)

TABLE III. Prevalence of CVLP Shedding by District of Residence

District	Sample size	% (no.) positive for CVLPs
Butha Buthe	56	67.9 (38)
Mafeteng	80	25.0 (20)
Leribe	77	20.8 (16)
Mohales Hoek	53	13.2 (7)
Total	266	30.5 (81)

alence in any of the other districts (67.9%). Butha Buthe was the first district to be sampled. The pattern of shedding was further broken down into week of shedding (Table IV). Shedding was highest in week 1 (67.9%) and declined every week to 10% in week 5. This comparison is confounded, however, by district of sampling changing during each of the first 4 weeks of sampling. In each of two districts, Mafeteng and Leribe, data were collected from four villages during weeks 2 and 3, respectively. During week 5, data from two additional villages in each of these districts were collected. Shedding was lower during week 5 than in week 2 in Mafeteng district ($P < 0.01$) and in week 5 compared to week 3 in Leribe district ($P < 0.01$).

Only one stool sample was taken from each child during October–November 1984. In an attempt to ascertain whether prolonged shedding of CVLPs occurred, stools were collected again from six children in March 1985, 5 months after initial collection. These children had been shedding large numbers of CVLPs during the first collection, but all six second stool samples were negative by EM.

The proportion of children shedding bacterial pathogens was as follows. For *Campylobacter* spp. it was 6.4%, for enteropathogenic *E. coli* 12.0%, for enterotoxigenic *E. coli* 4.7%, and for enteroinvasive *E. coli* 9.4%. There was no significant association between the rates of shedding of these bacterial pathogens and the presence or absence of CVLPs in the stool. In addition, the protozoan pathogen *Giardia lamblia* was present in 23.7% of stools and the protozoan *Entamoeba coli* in 17.7% [Esrey et al., 1988]. The rates of shedding of other parasites (other *Entamoeba* spp., *Hymenolepis spp.*, *Endolimax* spp., *Chilomastix* spp., and *Taenia* spp.) were below 3.0%. There was no significant association between the shedding of any of these parasites, or the total number of parasites detected in stools, and the presence or absence of CVLPs.

Similarly, CVLP shedding was not significantly related to any of the following variables, which have been defined in detail elsewhere [Esrey et al., 1987, 1988]: quantity of water used per household per day, exclusive use of the improved (standpipe) water supply, use of a latrine, degree of crowding in the home, quantity of household possessions, age of mother and marital status (data not shown).

DISCUSSION

In this paper we report the shedding of coronavirus-like particles by almost one-third of 266 children examined in Lesotho. The term *coronavirus-like particle* has been used by a number of authors, but not all of the particles described have been exactly alike. In one of the earliest reports on the presence of CVLPs in stool [Mathan et al., 1975], three different forms of fringe were described. Biochemical characterization of CVLPs to date has involved immunoblotting [Resta et al., 1985; Battaglia et al., 1985] and electrophoresis of proteins using sensitive silver staining [Schnagl et al., 1987], and much remains to be learned about the biochemical and antigenic make-up of CVLPs with different appearances.

In this study, at least two of the children were shedding particles with different appearances in the same stool. The significance of this finding is now known, and neither child was reported to have diarrhea. It is not known whether different "strains" of CVLP exist, but there is some evidence by immune electron microscopy that there may be antigenic variants [Schnagl et al., 1986]. One other line of evidence for more than one group of CVLPs came from the study in Gabon [Sitbon 1985]. Different seasonal variations in the shedding of CVLPs amongst the diarrheal and control groups indicated the existence of two or more groups of CVLPs with different epidemiological characteristics.

The shedding of CVLPs was not significantly associated with diarrhea. This is in agreement with Schnagl et al. [1978, 1979], who detected CVLPs in the stools of Australian aboriginal children with similar frequency whether healthy or with acute diarrhea. Moreover, Sitbon [1985] found a higher proportion of healthy children in Gabon shedding CVLPs (65.2%) than children with diarrhea (38.5%). Thus it is unlikely that CVLPs shed by children in developing populations are a major cause of diarrhea. Nevertheless, causation of diarrhea

TABLE IV. Prevalence of CVLP Shedding by Week and by Day of Stool Collection in 21 Rural Villages

Week/day of visit	District	Village	Sample size	% (no.) positive for CVLPs By day	By week
1 1	Butha Buthe	1	12	75.0 (9)	
1 2	Butha Buthe	2	6	83.3 (5)	67.9
1 3	Butha Buthe	3	25	60.0 (15)	
1 4	Butha Buthe	4	13	69.2 (9)	
2 1	Mafeteng	5	13	46.2 (6)	
2 2	Mafeteng	6	15	33.3 (5)	34.0
2 3	Mafeteng	7	6	50.0 (3)	
2 4	Mafeteng	8	16	18.8 (3)	
3 1	Leribe	9	17	17.6 (3)	
3 2	Leribe	10	12	16.7 (2)	24.6
3 3	Leribe	11	14	50.0 (7)	
3 4	Leribe	12	14	14.3 (2)	
4 1	Mohales Hoek	13	12	16.7 (2)	
4 2	Mohales Hoek	14	14	0.0 (0)	
4 2	Mohales Hoek	21	8	12.5 (1)	13.2
4 3	Mohales Hoek	15	9	33.3 (3)	
4 4	Mohales Hoek	16	10	10.0 (1)	
5 1	Leribe	17	12	16.7 (2)	
5 2	Leribe	18	8	0.0 (0)	10.0
5 3	Mafeteng	19	16	12.5 (2)	
5 4	Mafeteng	20	14	7.1 (1)	
Total		21	266	(81)	30.5

cannot be ruled out, as prolonged infection could occur, starting with diarrhea and resulting in reduced pathogenicity and hence tolerance.

Assuming that CVLPs are enteric viruses, spread could be by the fecal-oral and/or respiratory route. If the spread is fecal-oral, then transmission is most likely to be related to conditions of hygiene rather than to the quality of drinking water, because no association between CVLP shedding and quality of water was found [Esrey et al., 1987]. Twenty-five % of children shed at least one bacterial pathogen in this study, which is a further indication that hygiene standards were low. However, CVLP shedding was apparently unrelated to the amount of water used per household, the presence of a latrine, or characteristics of the mother that might be related to hygiene. Spread by the respiratory route may be possible, but CVLP shedding in this study was not related to crowding.

The present study was conducted in a temperate climate, and the rates of shedding were comparable to those reported for tropical countries [Mathan et al., 1975; Sitbon 1985]. Assuming that CVLPs are enteric viruses, it seems likely that transmission of these agents could occur in any climate, given the correct predisposing factors. It is not known what these factors might be. Poor hygiene and malnourishment may play a role. It is also possible that susceptible children such as those found in our study are effectively immunocompromised as a result of repeated exposure to pathogens. The figures reported by other authors, revealing high rates of shedding in some third world populations [Mathan et al., 1975; Sitbon, 1985; Schnagl et al., 1978, 1979] may also reflect exposure to multiple pathogens. Thus high rates of shedding in some populations may be more a result of environmental circumstances than an inherited susceptibility to such agents.

The increasing prevalence of CVLP shedding with increasing age up to 3–4 years in this study is of questionable significance. It might suggest chronic infection with a cumulative increase in the number of children infected from birth. The apparent drop in the proportion of children shedding CVLPs beyond the age of 4 years does not fit this possibility. Although we could not demonstrate prolonged shedding, chronic infection with seasonal shedding cannot be discounted. It seems more likely that the progressive decrease in the proportion of children shedding CVLPs over the 5-week study period was due to seasonal shedding. The results were confounded by geographical differences in the populations sampled in different weeks, but it was evident from studies done in the same districts at different times that the proportion of children shedding particles was decreasing significantly. The reason for such a pronounced drop in shedding rates between spring and summer is not understood.

The controversy over the viral nature of CVLPs [Dourmashkin et al., 1980] has been compounded by the recent study reported by Schnagl et al. [1987] on the protein profiles of CVLPs by polyacrylamide gel electrophoresis. At least 38 polypeptides were detected in highly purified preparations of Central Australian CVLPs, which is more than the number known for poxviruses, the most complex DNA viruses yet described. Thus, the question of whether or not CVLPs are viruses has still not been satisfactorily answered. These authors put forward some evidence that they are unlikely to be mycoplasmas, but did not rule out the possibility that they may be fragments of parasites. However, in

the present study there was no significant association between the shedding of CVLPs and infestation by either of the two most commonly found protozoa (*Giardia lamblia* and *Entamoeba coli*).

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