
Coronavirus-Like Particles in Adults in Melbourne, Australia

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Coronavirus-like particle(s) (CVLP) are faecal-derived pleomorphic membrane bound virus-like particles characterised by a fringe of club-shaped spikes that measure about 27 nm in length. The association of CVLP with a variety of social, clinical, and epidemiological factors was examined after a 69 month survey of faeces received for routine testing at an infectious diseases hospital. CVLP was found most commonly in three groups: first, intellectually retarded individuals who were usually inmates of institutions; second, recent overseas travellers who were either Indochinese refugees/immigrants or were overseas travellers who had usually visited developing communities for lengthy periods; and, third, male homosexuals who had a history of multiple sexual contacts and/or venereal disease. It was concluded that the excretion of CVLP had a strong association with unhygienic living or working conditions irrespective of any clinical symptoms the individual might show.

KEY WORDS: hygiene, faeces, virus-like particles

INTRODUCTION

Coronavirus-like particle(s) (CVLP) is the name applied to a group of pleomorphic membrane-bound virus-like particles characterised by a distinct fringe of spikes [Macnaughton and Davies, 1981]. CVLP are found in faeces, and negative-staining electron microscopy is the only reliable method for their detection [Macnaughton and Davies, 1981]. The precise nature, clinical significance, and epidemiology of CVLP are poorly understood [Macnaughton and Davies, 1981; Kidd et al., 1989].

Although CVLP are commonly detected in developing communities [Mathan et al., 1975; Marshall et al., 1982; Sitbon, 1985; Kidd et al., 1989], they appear less common in developed communities [Macnaughton and Davies, 1981]. However, there have been few detailed controlled surveys of CVLP in developed communities, and the precise relationship between CVLP excretion in developed and developing communities is not clear.

This study examines the question using adults in Melbourne, Australia, as the test population.

MATERIALS AND METHODS

Materials

Fairfield Infectious Diseases Hospital is the chief centre for the diagnosis and management of infectious diseases in the state of Victoria, Australia, and as such receives a wide range of specimens from a variety of individuals. During the course of a 69 month period from January, 1980, to September, 1985, over 2,000 faecal specimens from individuals aged 16 years and over were processed and examined by electron microscopy. These specimens were chiefly from patients with a history of gastroenteritis, although faecal specimens were also received from other patients and healthy persons temporarily resident in the hospital.

This study is based on the 34 cases found in the course of the survey when CVLP were detected and detailed records were available. In 26 of these patients, which are referred to as gastroenteritis cases, diarrhoea was a major symptom prior to admission. In the remaining eight cases, diarrhoea was either absent or only a minor symptom prior to admission. In seven of these eight nongastroenteritis cases, a variety of illnesses was recorded, including hepatitis (two cases), malaria, pneumonia, malignant mesothelioma, leprosy, and chest infection. The remaining nongastroenteritis case was apparently healthy.

A control group for the 26 gastroenteritis excretors of CVLP was chosen as follows: A control case was selected for each experimental case by using the first recorded individual after the experimental in the laboratory log book, who was 16 years old or more and who was within ± 5 years of age of the experimental, who had symptoms of diarrhoea prior to admission, for whom no CVLP were detected, and for whom detailed records were available. This control group therefore

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represents a random selection of gastroenteritis cases matched in age and time of collection to the CVLP-excreting gastroenteritis group. The two experimental groups and the control group represented exclusively inpatients at Fairfield Hospital.

Preparation of Faecal Samples

Faecal samples were processed as described by Oliver et al. [1985]. Briefly, faecal specimens were prepared as a 20% (w/v) suspension in Hank's complete balanced salt solution, vigorously shaken, then centrifuged twice at low speed to deposit debris. The clarified supernatant was then concentrated and further purified by ultracentrifugation through a sucrose cushion.

Negative-Staining Electron Microscopy

Initially the purified concentrated faecal specimens were examined after negative staining with 3% phosphotungstic acid (pH 7.0) on 400 mesh Formvar-carbon-coated grids. At least four grid squares were examined for each specimen using a Philips 301 electron microscope. Virus and virus-like particles were photographed and measured from photographic negatives. Catalase crystals, with half the lattice spacing taken to be 8.6 nm, were used as a calibration standard. In all positive specimens, CVLP numbered, on average, at least one particle per grid square.

In a further systematic study of the morphology of CVLP, a total of 815 randomly selected CVLP were photographed from 31 of the 34 individuals positive for CVLP. In each case, between 15 and 38 CVLP were photographed.

RESULTS

Morphology of CVLP

CVLP varied greatly in shape and size both within an individual and from individual to individual. They varied in shape from roughly round or ovoid to highly irregular (Fig. 1a). CVLP measured along their longest axis (excluding spikes) could vary from as little as 35 nm to more than 500 nm.

The variability in size of CVLP appears to arise from the capacity of larger CVLP to break up into smaller particles through the formation of constrictions (Fig. 1b). Particles with constrictions were seen in 15 of 31 cases studied in detail, although the actual incidence of these particles was low, with only 24 identified in 815 particles studied.

CVLP were recognised with confidence by virtue of their fringe of club shaped spikes (Figs. 1-4), which had a length of about 27 nm (mean \pm S.D. = 27.2 ± 6.0 nm, $n = 34$; measured from one spike from one particle from each of 34 individuals). Sometimes the appearance of the spikes was obscured by a fuzziness on the CVLP. The fuzziness could vary in intensity as well as in the proportion of spikes it covered (Fig. 2).

The morphology of the spikes varied both within an individual and from individual to individual, but there were two basic forms: spikes with one terminal bleb

and spikes with two terminal blebs (Fig. 3). A systematic analysis of 815 CVLP from 31 individuals indicated that CVLP with spikes with one bleb and CVLP with spikes with two blebs occurred in the same individual in 19 (61%) cases.

In 30 of 31 individuals, thread-like fibres were seen radiating from the fringe of spikes of some CVLP (Fig. 4). CVLP with fibres were noted in a total of 191 of 815 particles studied in detail. Thus CVLP have a varied morphology but can be defined as pleomorphic particles characterised by a fringe of spikes with an average length of about 27 nm. The spikes have a minimum of one terminal bleb and frequently exhibit a second bleb.

Classification of Individuals Excreting CVLP

CVLP excretors could be classified into six categories based on their recent social history (Table I).

Intellectually handicapped and institutionalised. Individuals who were intellectually handicapped and institutionalised made up about one-third of all CVLP excretors in the gastroenteritis group (Table I). Of these, five were from one institution, two were from a second institution, and one was from a third institution, indicating that CVLP excretion was not restricted to any one institution. Intellectually handicapped and institutionalised individuals were not found in the control group (Table I).

Intellectually handicapped and not institutionalised. One individual who was intellectually handicapped but not institutionalised was in the CVLP-excreting gastroenteritis group (Table I). This 60-year-old pensioner lived with her 90-year-old mother in a house described as being "in a dilapidated state."

Recent overseas travellers. The most common group excreting CVLP (both with and without gastroenteritis) was recently arrived overseas travellers i.e., those who had arrived from overseas within 3 months of being admitted to Fairfield Hospital (Table I). An examination of the travel itinerary of overseas travellers excreting CVLP and those travellers not excreting CVLP suggests that places visited and lifestyle are related to the excretion of CVLP, although total time overseas is not related.

The mean time spent in "developing communities" was significantly longer for travellers with gastroenteritis excreting CVLP, excluding Indochinese refugees/immigrants (mean \pm S.E. = 33 ± 11 weeks; $n = 7$) compared to travellers who were not excreting CVLP (mean \pm S.E. = 2 ± 1 weeks, $n = 5$) ($P < 0.05$, Student's t test). It is also notable that seven of the 19 overseas travellers excreting CVLP were Indochinese refugees/immigrants, whereas no such individuals were found in the control group. On the other hand, there was no significant difference in mean total time overseas between overseas travellers with gastroenteritis excreting CVLP excluding Indochinese refugees/immigrants (mean \pm S.E. = 49 ± 15 weeks; $n = 8$) and

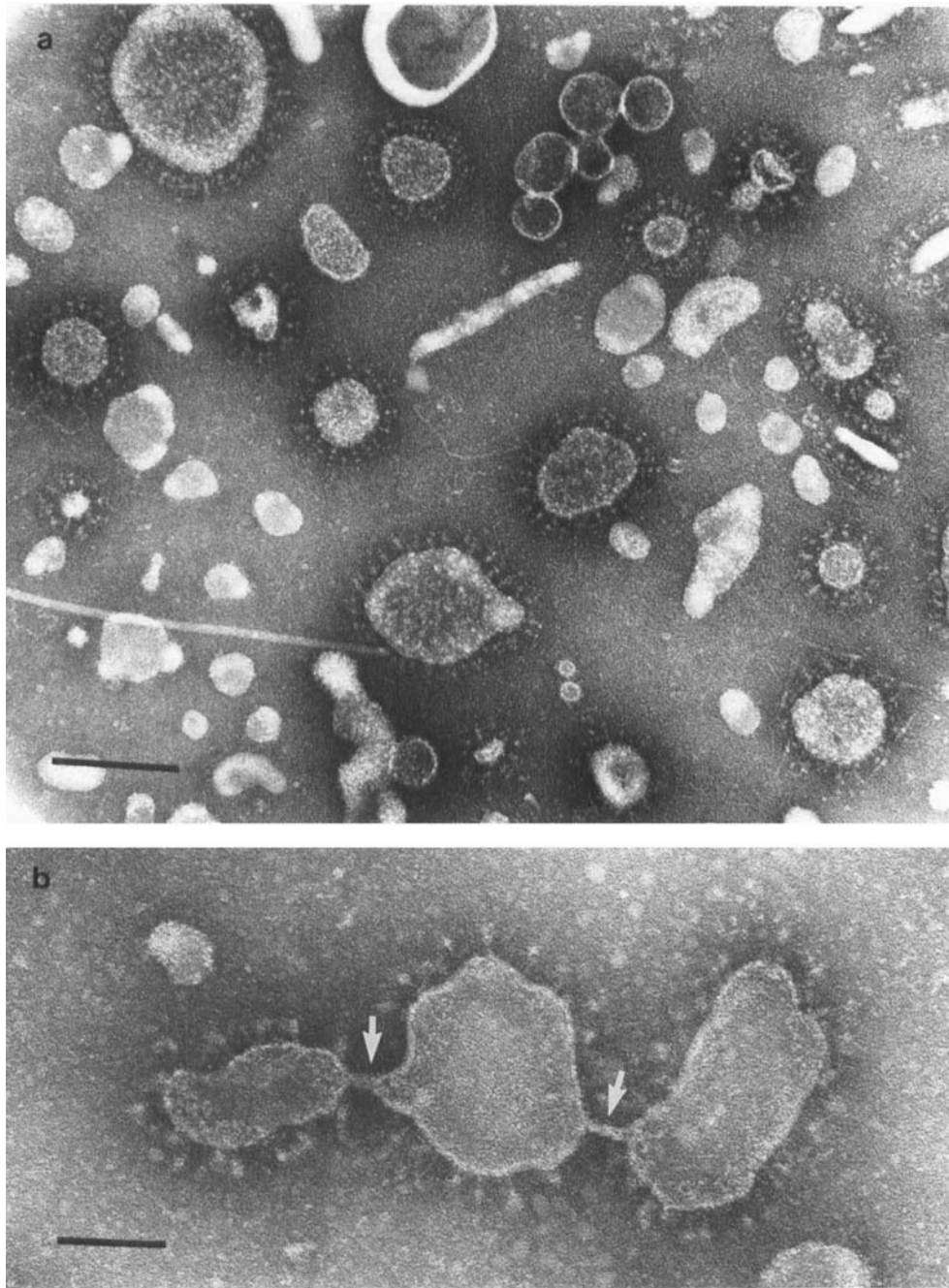


Fig. 1. Electron micrographs illustrating the variety of sizes and shapes of CVLP. **a:** Lower-power electron micrograph showing a variety of CVLP of different sizes and shapes in the same field. Bar =

200 nm. **b:** CVLP with two constrictions (arrows). The formation of such constrictions appears to be the method by which CVLP break up into smaller particles. Bar = 100 nm.

travellers not excreting CVLP (mean \pm S.E. = 12 ± 9 weeks; $n = 6$) ($P > 0.05$; Student's t test).

Health workers. One individual, a nurse, could be classified only in this category (Table I). Another nurse was noted in the gastroenteritis control group (Table I). Nothing in their respective histories gave any clue to how one may have become "infected" with CVLP but not the other.

It was also noted that a number of other individuals in the experimental and control groups were health care workers (Table I). These included two nurses in the group of 13 recent overseas travellers in the CVLP excreting gastroenteritis group, two nurses in the group of six recent overseas travellers in the gastroenteritis control group, and one nurse who had a history of multiple sexual contacts and/or venereal disease in

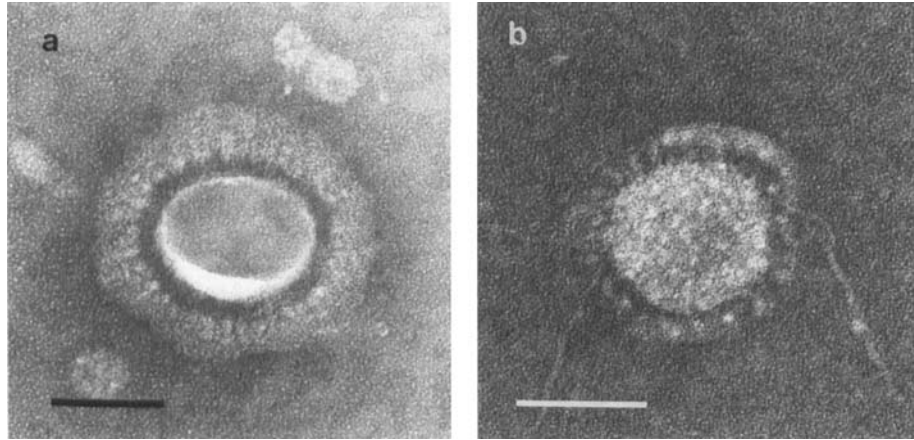


Fig. 2. Electron micrographs showing CVLP with heavy concentrations of fuzzy material on the fringe of spikes. The fuzzy material can cover the entire fringe (a) or only part of it (b). Bars = 100 nm.

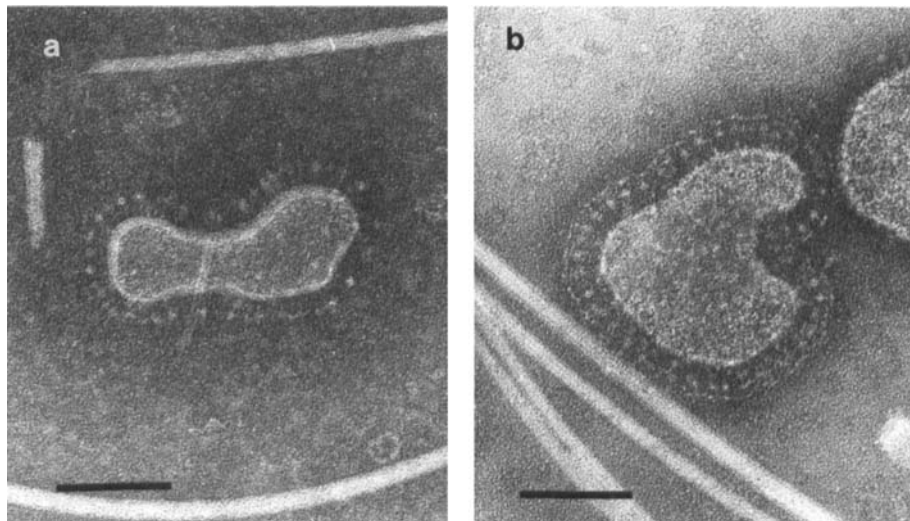


Fig. 3. Electron micrographs showing CVLP with spikes with one terminal bleb (a) and spikes with two terminal blebs (b). Bars = 100 nm.

the CVLP-excreting nongastroenteritis group (Table I). It must be concluded that as health care workers fall equally into both experimental (individuals with gastroenteritis excreting CVLP) and control (individuals with gastroenteritis not excreting CVLP) groups, being a health care worker is not in itself a risk factor for the excretion of CVLP.

Individuals with a history of multiple sexual contacts and/or venereal disease. Two individuals, both male homosexuals, belonged to this category in the CVLP excreting gastroenteritis group (Table I). One of them admitted to numerous sexual contacts and at a later admission was found to have antibody to HIV (human immunodeficiency virus). The second admitted to a past history of gonorrhoea. (In addition, one over-

seas traveller with gastroenteritis, a male airline steward who was excreting CVLP, was also noted to be a homosexual with a history of multiple sexual contacts and venereal disease). One individual belonging to this category was noted in the CVLP-excreting nongastroenteritis group (Table I). He was a homosexual and admitted having "contacts." All four individuals admitted to homosexual contacts within 6 months of admission.

One case belonging to this category was noted in the gastroenteritis control (no CVLP) group (Table I). An examination of this case history, however, points to a marked difference from those individuals excreting CVLP. This former female prostitute had, apparently, ceased these activities 3 years previously after migrat-

TABLE I. Classification of Individuals Excreting CVLP

Category	No. of individuals with gastroenteritis excreting CVLP ^a (experimental)	No. of individuals with gastroenteritis not excreting CVLP ^a (control)	No. of individuals without gastroenteritis excreting CVLP ^a (experimental)
Intellectually handicapped (institutionalised)	8 (31)	0	0
Intellectually handicapped (not institutionalised)	1 (4)	0	0
Overseas travel in 3 months prior to admission	13 ^{b,c} (50)	6 ^d (23)	6 (75)
Health worker	1 (4)	1 (4)	0
History of multiple sexual contacts and/or venereal disease	2 (8)	1 (4)	1 ^e (12)
Unclassified	1 (4)	18 (69)	1 (12)
Total	26 (100)	26 (100)	8 (100)

^aFigure in brackets gives percentage of total for that column.

^bTwo of these individuals were also nurses. One had worked in a refugee camp in Somalia while overseas and the other had worked for 2 days after arriving from overseas.

^cOne of these individuals was also a male homosexual with a history of multiple sexual contacts and venereal disease.

^dTwo of these individuals were also nurses by profession.

^eThis individual was also a nurse.

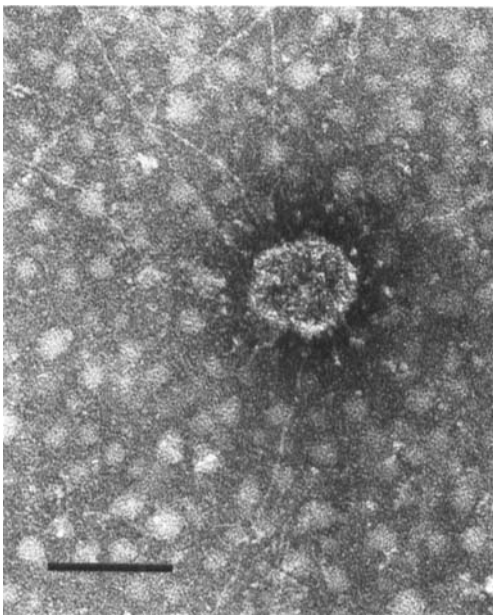


Fig. 4. Electron micrograph showing thread-like fibres associated with CVLP. Bar = 100 nm.

ing to Australia to marry. There was a suspicion of a past history of syphilis.

Unclassified. One individual (4%) of the CVLP excretors with gastroenteritis does not fall into any of the above five categories, compared to 69% in the control group (Table I).

Association Between "Poor Hygiene" and CVLP Excretion

In general, in the first five of the above categories, there is an obvious close association with "poor hygiene" over a period of time. In the histories of individuals in the unclassified category of the gastroenteritis control group, no such relationship with poor hygiene was noted. It can then be seen that 4% of CVLP excretors with gastroenteritis had no obvious association with poor hygiene compared to 69% of the control group. Statistical analysis showed that the difference between these two groups was highly significant ($P < 0.0005$; χ^2 test). In the CVLP-excreting nongastroenteritis group, only one of eight individuals had no obvious association with poor hygiene (Table I).

DISCUSSION

Although the morphology of CVLP is well documented [Macnaughton and Davies, 1981], a number of novel observations in this study contribute to an understanding of the variability of morphology of CVLP. First, smaller particles appear to arise through the formation of constrictions in larger particles. Second, particles with spikes with one bleb and particles with spikes with two blebs quite commonly occur in the same individual. It is possible the outer bleb has a tendency to break off, resulting in the formation of one blebbed spikes.

The general description of CVLP in this study accords closely to the general description of CVLP in other reports [Macnaughton and Davies, 1981]. The occasional finding of fuzzy material on CVLP seen in this

study may represent antibody on the particles. The frequent observation of fine thread-like fibres on many CVLP found in this study has not been previously noted. These fibres could represent thread-like debris that have adhered to the CVLP.

The results of this survey show that excretors of CVLP in Melbourne generally belong to three groups: intellectually handicapped individuals, recent overseas travellers who had spent some time in developing communities, and some homosexuals. The intellectually handicapped individuals excreting CVLP were mainly inmates of institutions. The recent overseas travellers excreting CVLP were commonly refugees or immigrants from developing communities or were overseas travellers who had visited developing communities for lengthy periods. The homosexuals excreting CVLP were all male and had a history of multiple sexual contacts and/or venereal disease.

The findings of a number of studies of CVLP in special population groups in developed communities are consistent with the general conclusions of this study. Both Kern et al. [1985] and Riordan et al. [1986] noted CVLP quite commonly in male homosexuals, some of whom developed AIDS. Moore et al. [1977] detected CVLP quite frequently in a centre for the intellectually retarded.

Although the findings of this study do not exclude the possibility that "infection" with CVLP may cause gastroenteritis, the results show that CVLP excretion and poor hygiene are closely related, irrespective of the clinical symptoms of the individual excreting the particles. This appears to be the factor linking all excretors of CVLP in developed and developing communities alike.

The nature and clinical significance of CVLP are controversial. Although the morphology of these particles is consistent with that of an enveloped virus, biochemical and serological studies, to date, appear confusing [Gerna et al., 1985; Mortensen et al., 1985; Resta et al., 1985; Battaglia et al., 1987; Schnagl et al., 1987]. The results of this study should aid further work by pinpointing the precise morphology of CVLP and the groups most likely to be found excreting the particles.

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