Yanagihara R:

The most widely available technique for serodiagnosis is immunofluorescence, using as targets hantavirus-infected Vero E6 cells which are fixed with acetone. There are other methods to determine the presence of antibody. These include hemagglutination-inhibition and plaque-reduction neutralization. Immunofluorescence as a method for viral diagnosis depends on the experience of the reader. We have found that most human sera with fluorescent antibody titers of 16-32, and even sometimes 64, do not contain neutralizing activity to hantaviruses. Therefore, we are reluctant to call these true positives. It is well-known that, even 20 and 30 years after getting hemorrhagic fever with renal syndrome, neutralizing antibody can be detected, and, frequently, it is much higher than the fluorescent antibody titers. Serodiagnosis by plaque-reduction neutralization tests is very specific, as there is no cross-neutralization between hantaviruses and other bunya- or bunya-like viruses.

Purtilo D (University of Nebraska, Omaha):

I just heard from Tom and Luis that it's unlikely that hemorrhagic fever is going to occur in Southern California; because, as you showed, the laboratory rats are being reservoired, and, here in Southern California, I understand they're using lawyers as experimental animals rather than white rats, because the antivivisectionist movement is very strong here, and there are more lawyers here than there are white rats, and they're cheaper. The other thing is that you can get them to do things that white rats won't do.

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# NATURAL HISTORY OF VIRUSES OF THE INTESTINE IN CONTRASTING ECOSYSTEMS: IMPLICATIONS FOR HEALTH

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## INTRODUCTION

Many different viruses have the capacity for infecting and replicating in micro-habitats of the intestinal tract. Eons of interaction between intestinal viruses and the intestinal mucosa have lead to a variety of host-parasite interrelationships, most of which are superficially known or hitherto unsuspected. Our knowledge has been determined in great part by advances in biotechnology and its capacity to recognize and study such viruses. On the other hand, changes in scientific interest and in funding have shifted research interest from the enteroviruses to the rotaviruses at a point where our understanding of the first was still insufficient. This is unfortunate because information for contrasting ecosystems, for instance, within developing countries is even more incomplete than for industrial countries. Enteroviruses and adenoviruses still play an important role in disease causation everywhere.

From an evolutionary perspective, the intestinal tract can be considered an invagination of external integuments, and, therefore, thousands of years of evolutionary adaptations led to specialization and development of myriads of bacteria, protozoa, yeasts and viruses in the intestinal habitats. Such changes explain the varying pathogenicity of agents and, in some instances, their coexistence within the human host.

Understandably, viruses have adapted less effectively than indigenous or autochthonous bacteria, and their pathogenicity is, thus, greater when contrasted with the harmless nature or, at worst, amphibiontic behavior of the indigenous flora. The high prevalence of enterovirus infection in poor populations reported by Parks et al. (1967) and Mata (1978) represent transient infections without persistence of any given serotype.

The pathogenic potential of some echoviruses and other enteroviruses is low compared to that of the rotaviruses, but not as low as that of many virus particles which are identified by electron microscopy (EM) in the absence of clinical manifestations (Madeley, 1986).

## GROUPS OF VIRUSES OF THE INTESTINE

Theoretically, any virus observable or isolated from intestinal materials (juices, cells, bolus, feces) might be considered an intestinal virus. This

**TABLE 1**

**GROUPS OF VIRUSES OF THE HUMAN INTESTINE**

| Group | Total No. of Serotypes | Causes Diarrhea |
|---|---|---|
| Rotavirus | 4 | yes |
| Pararotavirus | ? | ? |
| Adenovirus, fastidious | 2 | yes |
| cultivatable | 39 | some yes |
| Norwalk, Norwalk-like | 3 | yes |
| Astrovirus | ? | yes |
| Calicivirus | ? | yes |
| "Small round viruses" | ? | ? |
| Enterovirus, cultivatable | 63 | yes |
| fastidious | 5 | some yes |
| Coronavirus | ? | yes, chronic |

stringent definition would pose difficulty inasmuch as respiratory viruses are swallowed and then can be found in the intestine. On the other hand, viruses replicating in inflammatory cells, giant cells and lymphocytes that are shed into the intestinal lumen - for instance, measles virus and human immunodeficiency virus - could be recovered from the intestine without belonging there. More realistically, viruses of the intestine are only those that replicate preferentially, and usually abundantly, in intestinal mucosal cells and in adjacent lymphoid tissue, and are then shed in the stools, which represents the primary source for infection of human beings.

Table 1 lists the main groups of viruses of the intestine of human beings. The term "fastidious" is used by Madeley (1986) and others for adenoviruses which are difficult or yet impossible to grow in conventional cell cultures; it is applied here to enteroviruses which are difficult to isolate, for instance, enterovirus 70 (EV 70) or the agent of hemorrhagic conjunctivitis, and EV 72, a proposed agent of hepatitis A. Of the viruses listed in Table 1, rotaviruses, fastidious adenoviruses, and Norwalk and Norwalk-like agents are the most pathogenic, and have the capacity to induce diarrhea. Enteroviruses are typical intestinal viruses, easily isolated from stools by conventional techniques, and are clearly related to respiratory diseases and, to a lesser extent, diarrheal diseases. The non-fastidious (or cultivatable) adenoviruses are frequent agents of respiratory disease and can also induce diarrhea.

Several reports linking enteroviruses and non-fastidious adenoviruses to diarrheal disease were published in the late 1950's and 1960's when those viruses were fashionable. However, it turned out to be difficult to show an association

between them and diarrhea in view of their very low pathogenicity and the fact that most infections are asymptomatic or accompanied by subtle clinical signs (despondency, weakness, irritability or mild anorexia).

Statistical demonstration of an association of these viruses with diarrheal disease relies on the fortuitous occurrence of outbreaks involving virulent strains in populations with a high level of hygiene. Such circumstances have favored demonstration of virulent enteric viruses in the community because, with prevailing low rates of viruses, an excess of infections can be found associated with diarrhea and other clinical entities.

The situation in developing countries is quite different in that viruses of the intestine are so prevalent that they practically constitute an "indigenous viral flora" (Mata, 1978). This makes it difficult, if not impossible, to demonstrate that cultivatable enteroviruses and adenoviruses play a role in the causality of diarrhea; it also might explain conflicting results on diarrhea causality reported by different laboratories (Ramos-Alvarez and Olarte, 1964; Yow et al. 1963).

## DIVERSITY OF VIRUSES OF THE INTESTINE

Current knowledge on groups, subgroups and serotypes of viruses of the intestine is limited by available technology. Undoubtedly, new viruses will be discovered in the future to account for additional "non-specific diarrheas," still representing from 20 to 60% of the cases seen in the general population.

### Rotaviruses

These RNA viruses, named to remember their wheel-like (from Latin "rota") appearance (Flewett et al. 1973), measure about 65-75 nm at the outer capside (Holmes, 1983). Extensive immunologic and molecular studies in several laboratories have revealed the antigenic complexity of the rotaviruses. Two subgroups and four serotypes of human rotaviruses are currently recognized (Table 2). There is great genomic and antigenic variability, as revealed, for instance, in changes in rotavirus electropherotypes over time in any given community (Estes et al. 1985). Rotavirus electrophoretic variability possibly reflects genetic reassortment between different viruses infecting and replicating simultaneously in the same cells.

Strains of serotype 1 seem to be the most important clinically. Strains of serotypes 1 and 2 have been recovered from humans only. Serotype 3 contains strains of human, simian and porcine origin (Holmes, 1983).

### Adenoviruses

These DNA viruses have icosahedral symmetry and measure about 60 to 90 nm (Ginsberg, 1980). The cultivatable adenoviruses are found frequently associated with respiratory illnesses, but they are also recovered from feces and may be

**TABLE 2**

**ROTAVIRUS SEROTYPES FOUND IN HUMANS**

| Serotype[a] | Source of Isolation | Strains | Subgroup | RNA Pattern |
|---|---|---|---|---|
| 1 | Humans | Wa, KU, K8, DB | 2 | long |
| 2 | Humans | DS-1, S2, KUN, HN-126, 390 | 1 | short |
| 3 | Humans | M, P, YO, 14, 15, McM2, MO, Ito, Nemoto | 2 | long |
| | Animals | Simian MMU18006, SA11 | 1 | long |
| | | Canine CU-1 | 1 | long |
| | | Equine H2 | | long |
| 4 | Humans | St. Thomas 4, Hochi, Hosokawa | 2 | long |
| | Animals | Porcine Gottfried, SB-1, SB-2 | 2 | long |

[a] Serotypes 5 to 9 have been isolated from animals only.
Adapted from Estes et al. (1985).

related to gastrointestinal symptoms. There are 34 recognized adenovirus serotypes and seven additional candidates have been proposed (Table 3) (Madeley, 1986). Types 1, 2, 5 and 6 are found endemic or associated with sporadic cases of illness; they do not usually occur in outbreaks. Types 3, 4 and 7 are frequently epidemic. Type 8 is an agent of epidemic conjunctivitis, and type 9 and successive serotypes are recovered mostly from stools of asymptomatic persons, sporadic cases of respiratory or diarrheal disease, or occasional outbreaks of these illnesses.

Examination of fecal preparations by EM often fails to reveal adenovirus particles in persons from whom adenoviruses are isolated in conventional cell culture. On the other hand, EM often reveals typical adenovirus particles without a concomitant isolation of adenovirus in tissue culture (Madeley, 1986).

Serotypes 34 to 39 are rare, and serotypes 40 and 41 have been proposed as agents of diarrheal disease (de Long et al. 1983; Moritsugu, 1969; Uhnoo et al. 1983; Wadell et al. 1980). Demonstration of pathogenicity of fastidious adenoviruses has been complicated by the prolonged excretion period, which accounts for the frequent finding of virus without clinical manifestations. This is also the case of cultivatable strains with proclivity towards chronicity, although their low virulence probably is a more important determinant.

Norwalk and Norwalk-like Agents

These particles, measuring about 26-27 nm, comprise at least three related - but likely different - serotypes (Wyatt and Kapikian, 1981). The classical one

TABLE 3

ADENOVIRUS SEROTYPES OF HUMANS

| Serotype | Source[a] |
|---|---|
| 1-7 | feces, nasopharynx, eye |
| 8 | feces, eye |
| 9-33 | feces |
| 34 | urine, lung (transplant) |
| 35 | kidney, lung (immunosuppressed) |
| 36 | feces |
| 37 | eye, cervix |
| 38 | feces (?) |
| 39 | feces, nasopharynx |
| 40 | feces |
| 41 | feces |

[a] Cultivatable adenoviruses have been isolated from urine, nasopharynx and other sites in addition to those shown here.

Adapted from Madeley (1986).

is the Norwalk agent recognized by EM in a filtrate of diarrhea stools of a volunteer who ingested material from an outbreak in Norwalk. A similar agent discovered in Montgomery was found related to the Norwalk agent, while another found in Hawaii - although of similar size and structure - seemed different. Similar agents were discovered in two outbreaks in England (strains Ditchling and W), and while they were found related to each other, they were different from the Norwalk agent (Wyatt and Kapikian, 1981). Additional Norwalk-like viruses are likely to be recognized in the near future.

Enteroviruses

The human enteroviruses belong to the picornaviruses and contain RNA; they comprise three serotypes of polioviruses, 29 serotypes of coxsackieviruses, and 31 serotypes of echoviruses. Five additional serotypes have been described more recently, EV 68, EV 69, EV 70, EV 71 and EV 72 (Melnick, 1985; Wyatt and Kapikian, 1981). EV 70 is the cause of hemorrhagic conjunctivitis; EV 72 is the proposed agent of hepatitis A, a classification debated by some authors. Infection with the virus of hepatitis A can be associated with gastrointestinal symptoms in its initial phase.

Other Viruses

There is little or no knowledge on the antigenic diversity of astroviruses, caliciviruses, "small round" viruses and coronaviruses found in humans with or without diarrheal disease. Such information will be obtained when we learn about better ways to grow them in the laboratory (Wyatt and Kapikian, 1981).

## NATURAL HISTORY OF INFECTION

There are various ways to study the natural history of infection with viruses of the intestine. One way is by a prevalence survey of the general population; another, more difficult but more informative, is by long-term prospective study of populations in their ecosystem. In this case, the approach can be either the study of institutionalized children as they encounter illnesses, as in the Junior Village Study (Bell et al. 1961), children in the community during health and disease, as in the Cauque Study (Mata, 1978), or whole families in urban centers, as in the Virus Watch Programs in New York and Seattle or in the Tecumseh population (Fox et al. 1969, 1980; Monto et al. 1970).

Prospective studies search for agents when illnesses are reported or discovered (Fox et al. 1969) or at periodic intervals regardless of whether there is an illness or not (Mata, 1978). While this approach is more onerous, it provides unique information on the frequency of infection in the community, and on possible associations with particular diseases.

Viral isolation and demonstration of agents by EM or immunologic techniques can be complemented by serologic studies at specified age intervals, to show sero-conversion to a particular agent. However, this approach may be difficult or impossible to carry out in developing countries where there is prejudice or cultural barriers against collection of sequential blood specimens. On the other hand, cross-sectional serologic surveys of the general population may provide an adequate view of the rate of infection with viruses of the intestine, as reflected in the rate of sero-conversion to those viruses.

### Epidemiologic Considerations

Infection with enteroviruses, adenoviruses and rotaviruses is greatly in excess in developing countries (Mata, 1978) when compared with those observed in industrial countries (Gelfand et al. 1963). This is understandable in view of the large amounts of viruses shed in feces and nasal, oral and pharyngeal secretions. Contamination of drinking water is also important, and according to studies carried out in industrialized countries, infective viruses are recovered in large quantities from sewers and water of rivers, lakes and oceans (Melnick, 1985; Rao et al. 1986). The problem is likely worse in less developed countries, where drinking water is frequently contaminated with feces.

A possible influence of climate *per se* must be ruled out since rates of infection are high among institutionalized children in temperate climates who live under deficient sanitary conditions (Bell et al. 1961). On the other hand, the rate of infection with rotaviruses was found to be very low among rural children of Puriscal, Costa Rica, where personal hygiene and environmental sanitation are adequate and where wide separation between homes probably discourages the spread of many infections (Simhon et al. 1985). Thus, differences

in rates of infection between industrial and developing countries, in all probability, reflect differences in personal hygiene and sanitation rather than in climate or other host attributes such as the nutritional state.

Acquisition of Viruses by Neonates

The high rates of infection in developing countries reflect greater exposure to contaminated food and water, and to contaminated hands, objects, or even the breast (Wyatt and Mata, 1969). Exposure of the mouth of the child to viruses commences at birth for children living in deprived conditions. For instance, the Cauque Study showed that childbirth by squatting or kneeling is commonly associated with soiling of the newborn with maternal feces; rural women in traditional societies often carry viruses and other parasites (Mata, 1978). It was not unusual to find babies excreting enteroviruses from the very first few days of life (Table 4). Viruses were present in such high concentration as to suggest that they were replicating in the child's intestinal tract. These early neonatal infections are, for the most part, asymptomatic, owing to high amounts of secretory immunoglobulin A (S-IgA) and other antibodies in colostrum and milk (Wyatt et al. 1972). Infants were placed at the breast of a foster mother immediately after birth, and as soon as the mother secreted colostrum and milk, these were given to them (Mata, 1978).

On the other hand, infection with rotaviruses among rural neonates was very rare in a rural highland village of Guatemala (Mata et al. 1983a) and in highland

TABLE 4

ENTEROVIRUSES IN MECONIUM AND FECES OF GUATEMALAN INDIAN NEONATES, FIRST THREE DAYS OF LIFE, 1964-1969

| Day of Life | Number of Children Tested | Number Positive (%) | Virus Type Isolated | Viral Concentration[a] |
|---|---|---|---|---|
| 1 | 79 | 1(1.3) | echo 7 | 2 |
| 2 | 54 | 4(7.4) | echo 6 | 3 |
| | | | echo 6 | 3 |
| | | | echo 6 | 3 |
| | | | polio 1 | 5 |
| 3 | 61 | 5(8.2) | polio 1 | 4 |
| | | | echo 6 | |
| | | | echo 9 | 5 |
| | | | echo 11 | 5 |
| | | | echo 7 | 5 |
| | | | echo 6 | 5 |

[a] log of $TCID_{50}$/gr.

Adapted from Mata (1982a).

populations (both urban and rural) of Costa Rica (Simhon et al. 1985). The findings contrast with those in neonates of industrialized urban centers, among whom early neonatal rotavirus infection is common (Bishop et al. 1979; Chrystie et al. 1978; Perez-Shael et al. 1984).

Enteroviruses in Infancy and Early Childhood

The description of the natural history of virus infection was based on the long-term prospective study in the Guatemalan village (Mata, 1978). Children were progressively infected with enteroviruses and they were weaned; infection rates declined after completion of weaning, in this community around the second year of life, suggesting the acquisition of immunity. To illustrate the acquisition of those viruses in the first year of life, Fig. 1 shows the pattern of shedding of enteroviruses and cultivatable adenoviruses among 18 infants selected at random among a cohort of 45 children (Mata, 1978).

Extracts of stool samples collected each week from these children were cultured in primary human amnion and kidney and in HEp-2 cells, and the isolates were grouped according to cell specificity and cytopathic effect. Some children were relatively clean during the first trimester of life, while some were infected shortly after birth, despite the fact that they were exclusively breastfed, as is customary in Mayan Indian villages. The rate of adenovirus infection is likely an underestimate because enteroviruses having a faster replication cycle, they interfered with the growth of adenoviruses in tissue culture. The monumental force of infection can be appreciated in Table 5, where rates of excretion of enteroviruses and adenoviruses are shown, by semester, for the whole cohort of 45 children. What is evident is that about one half of all infants shed viruses by the end of the first year of life, and the rate was 60% by the end of the third year, when most children had been weaned.

The Cauque Study also permitted observations on the acquisition of wild poliovirus since it was conducted during an epoch in which there was not yet a national polio immunization program. Table 6 shows that 47% of infants excreted at least one type of poliovirus during the first year of life.

The rates of infection described possibly reflected many multiple viral infections. This aspect, not investigated in the Cauque Study, had been explored by Parks and co-workers (1967) in the Karachi Study, a unique research not yet repeated. These researchers re-tested in tissue culture a set of virus-positive specimens, after neutralization of the identified virus with specific antiserum, in order to discover another possible hidden virus (or viruses). Indeed, the study showed infections with two, three, and more than three viruses (Table 7). Of 59 virus-positive specimens obtained from children with diarrhea, 51% were found with at least two viruses and 10% with three viruses or more. Among 57 virus-positive specimens from children without diarrhea, 40% had at least two

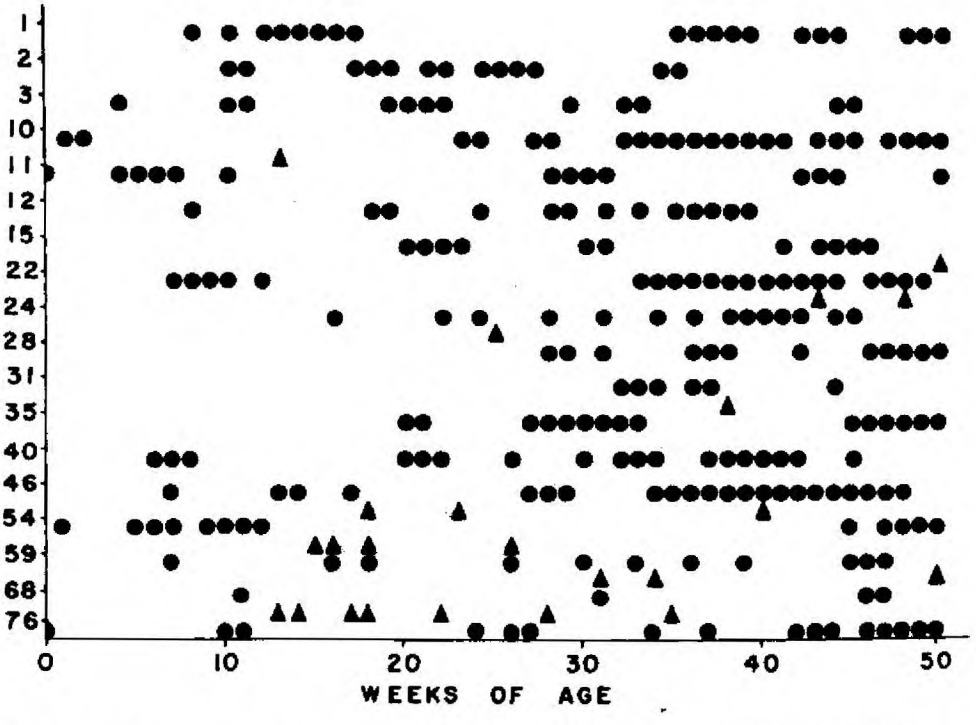


Fig. 1. Enteroviruses (solid circles) and adenoviruses (solid triangles) excreted by 18 children of the cohort of 45 during the first year of life. All infants were almost exclusively breast-fed for four to seven months, and supplementation with village foods was minimal during the first year of life. Breast feeding, in this culture, continues for two to three years. Some children were relatively free of virus infection in the first few months of life; other infants were infected shortly after birth. By the end of the first year of life, most children were excreting enteroviruses at any time they were sampled.
From Mata (1978).

viruses and 18% at least three; there were no significant differences between the two groups of specimens. The Karachi Study also showed, as did the Cauque Study, that viruses were excreted in large amounts, 20 to 5,000 $TCID_{50}$ for polioviruses, and 20 to 90,000 $TCID_{50}$ for coxsackieviruses (Parks et al. 1967).

TABLE 5

FECAL EXCRETION OF ENTEROVIRUSES AND ADENOVIRUSES BY AGE IN A COHORT OF 45 CHILDREN, FROM BIRTH TO THREE YEARS OF AGE, 1964–1969

| Age, Months | Number of Specimens | Enteroviruses | Adenoviruses |
|---|---|---|---|
| 0–5 | 1,116 | 230(20.6)[a] | 34(3.1) |
| 6–11 | 1,162 | 483(41.6) | 46(3.9) |
| 12–17 | 917 | 481(52.5) | 33(3.6) |
| 18–23 | 953 | 438(45.9) | 60(6.3) |
| 24–29 | 908 | 446(49.1) | 58(6.4) |
| 30–35 | 867 | 530(61.6) | 48(5.5) |

[a] Number of positive specimens (percentage).

Adapted from Mata (1978).

TABLE 6

FECAL EXCRETION OF WILD POLIOVIRUSES IN THE FIRST YEAR OF LIFE IN A COHORT OF 45 INFANTS EXAMINED WEEKLY, 1964–1969

| Poliovirus Serotype | Number of Children Positive (Percentage) |
|---|---|
| 1 | 7(15.6) |
| 2 | 5(11.1) |
| 3 | 3(6.7) |
| 1 and 2 | 2(4.4) |
| 1 and 3 | 1(2.2) |
| 2 and 3 | 2(4.4) |
| 1, 2 and 3 | 1(2.2) |
| Grand total | 21(46.7) |
| Total with: | |
| 1 | 11(24.4) |
| 2 | 10(22.2) |
| 3 | 7(15.6) |

Adapted from Mata (1982a).

TABLE 7

MULTIPLE ENTEROVIRUS IN VIRUS-POSITIVE SPECIMENS, KARACHI STUDY

| Diarrhea | Number of Specimens Retested | Number of Virus Isolates 2 | 3 | 4 |
|---|---|---|---|---|
| With | 59 | 30(51)[a] | 6(10) | 0 |
| Without | 57 | 23(40) | 10(18) | 2(4) |

[a] Number positive (relative percentage).

Adapted from Parks et al. (1967).

The large infectious dose passed into the environment is compounded by the chronicity of some infections. While more needs to be learned about this regard, it is widely accepted that about one-half of the enterovirus infections last for more than one week, as shown for polioviruses in the Cauque Study (Table 8) (Mata, 1978). The situation might be different in older children, especially if there is malnutrition, since it is known that malnourished children have impaired immunity.

Rotaviruses in Infancy and Early Childhood

The description of the natural history of rotavirus infection in contrasting populations was based on data sets from two field studies in Central America: the Cauque Study, conducted in 1964-1969 in a Guatemalan Indian rural village

TABLE 8

DURATION OF FECAL EXCRETION OF POLIOVIRUS IN THE FIRST YEAR OF LIFE IN A COHORT OF 45 CHILDREN CULTURED WEEKLY FROM BIRTH, 1964-1969

| Duration Weeks[a] | Poliovirus Serotype: 1 | 2 | 3 |
|---|---|---|---|
| 1 | 7(54)[b] | 3(38) | 4(50) |
| 2 | 3(23) | 3(28) | 1(13) |
| 3 | 1(8) | 1(9) | 1(13) |
| 4 | 1(8) | 3(28) | 1(13) |
| 5 | 0 | 0 | 1(13) |
| 6 | 1(8) | 1(9) | 0 |
| Total | 13 | 11 | 8 |

[a] Two episodes of virus shedding were considered different if they were separated by three or more negative weeks.

[b] Number of episodes (relative percentage).

Adapted from Mata (1978).

(Mata, 1978); and the Puriscal Study, carried out in a Costa Rican rural mestizo complex of villages (Mata, 1982a).

The two populations were located at a similar altitude (from 500 to 1,800 meters above sea level). Both are rural, although the degree of "ruralism" was considerably greater in Puriscal than in Cauque, with large distances separating households of more than one half of the people. Both populations were poor, but poverty of Puriscal was compensated to a great extent by a significantly higher level of education, personal hygiene, environmental sanitation and availability of health services (Mata, 1982a, 1983a). The contrast with the village of Cauque is striking since the latter did not have (and still does not have) adequate sanitation and health services, while education and personal hygiene remain deficient.

Both investigations had similar study design and employed similar field methodology. Fecal specimens were collected every week from each child in cohorts of similar size. Collateral clinical and nutritional information was also obtained in both places. The specimens of the Cauque Study, collected during the period 1964-1969, had been preserved at -70°F for 13 to 18 years. This unique collection of samples was compared with the also exceptional collection of Puriscal specimens, collected during 1981-1984 (Simhon et al. 1985). All specimens were tested for rotavirus antigen, in duplicate, by an enzyme-linked immunosorbent assay (ELISA) (Mata et al. 1983a). While the ELISA used was the same, the opportunity to diagnose rotaviruses was enchanced for the Puriscal specimens by centrifugation at 200,000 x g for 60 minutes (Simhon et al. 1985).

Rotaviruses in Cauque

All children in this village excreted rotaviruses at least once during the first three years of life. The complete data on these viruses for 45 cohort children are depicted in Fig. 2. Individual children are shown in rows, arranged according to date of birth. While children were observed during their first three years of life, the total period of observation was six years, as chidren were recruited over a span of two years. The years and months of the study are shown at the top of the figure.

There were wide differences in virus shedding among the children: one child had only one rotavirus infection, fifteen had two, twelve had three, twelve had four, four had five, and one child had seven infections (Mata et al. 1983a). Rotavirus infections did not persist, and of the 142 infections recorded in the cohort, 116 lasted for only one week, 14 lasted for two weeks, six lasted three weeks, four lasted four weeks, and two lasted five weeks. It is possible that more sensitive techniques developed in the future - for instance, a better system for virus isolation - will reveal prolonged infections.

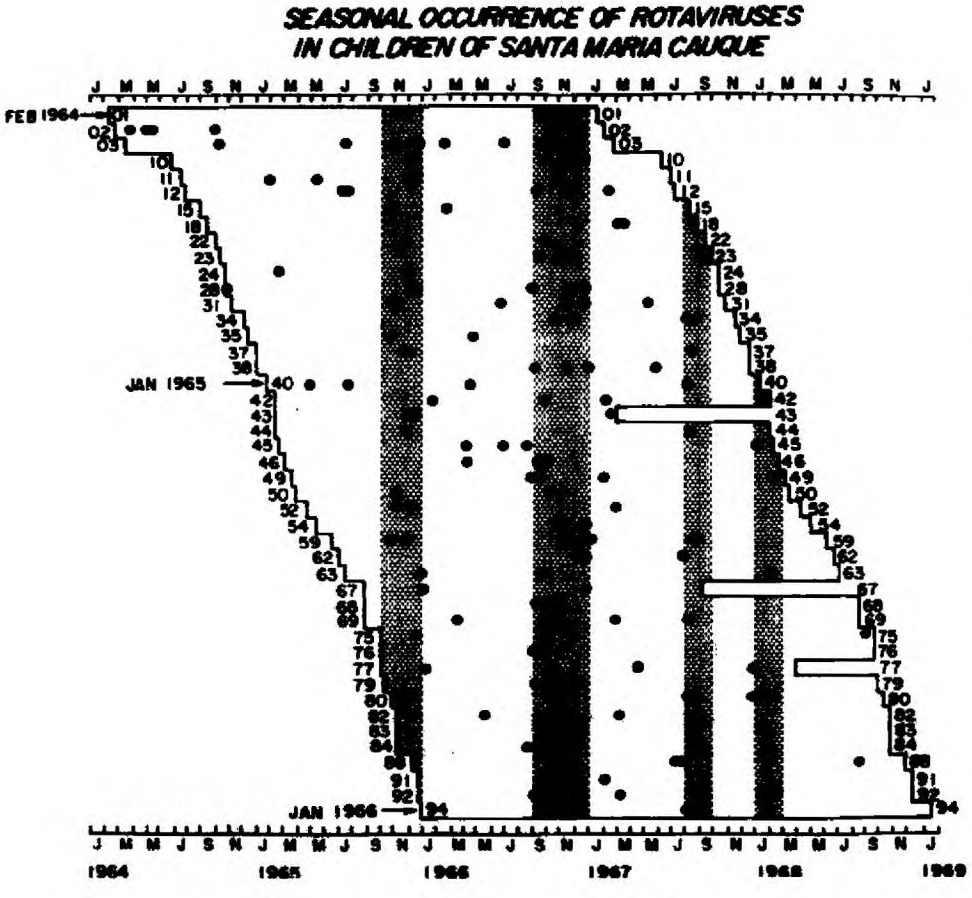


Fig. 2. Natural history of rotavirus infection in a cohort of 45 children from a Guatemalan rural village followed from birth to three years of age. Each dot represents one week positive for rotavirus by ELISA. The numbers of children are at the left, and the year and month are indicated at the top and bottom of the figure. Two children died and one child withdrew from the study before completing three years (children 67, 77 and 43, respectively).
From Mata et al. (1983a).

The overall incidence of rotavirus was 2.41 per 100 child-weeks or 1.25 per child-year. Rates were lower in the first six months of life, when breast feeding was intense and almost exclusive. Thereafter, incidence rose and was greater during epidemic periods, as shown with shaded areas in Fig. 2. Four village epidemics were observed during the study period. The first occurred in November and December of 1965, with rates of 29% and 25% of children infected,

respectively. November through February are relatively cold and dry months in the Guatemalan highlands. September through December of 1966 were also epidemic, and rates fluctuated between 18 and 44%. Rates were around 40% in August of 1967. In January and February of 1968 - when all cohort children were more than two years old - rates were still high, about 20% (Mata et al. 1983a).

Rotaviruses in Puriscal

Most infants are breast-fed in this region as a result of "rooming-in" and promotion of nursing, effected concomitantly with the study (Mata et al. 1981). Breast-feeding, however, was not as exclusive and intense as it was in the traditional population of Cauque.

The display of all viral diagnosis in Puriscal children is in Fig. 3. Clearly, there is great contrast with the data for Cauque, in that the number of infections was considerably less in Puriscal than in Cauque (Simhon et al. 1985). There were no marked differences in age distribution of rotaviruses in Puriscal. Considering that Puriscal children were observed for only two years instead of the three of Cauque, 63% excreted rotaviruses during the study period. The number of infections per child was also significantly less in Puriscal than in Cauque. Seven (14%) children experienced reinfections, separated by periods of 4 to 74 weeks. There were 39 independent infections for an overall incidence of 1.05 per 100 child-weeks or 0.5 per child-year, considerably less than in Cauque. As in the Guatemalan village, rotaviruses were endemic in Puriscal throughout the study period, but children excreted more viruses during the cooler and drier months (December through February), as in Cauque. February and October of 1982, and February and December of 1983 were peak months.

Rotavirus electropherotypes were studied by Simhon et al. (1985) for selected Costa Rican specimens, and almost all strains from diarrhea and healthy cases were found to belong to the "long" RNA pattern. There was little electrophoretic diversity, and only five electropherotypes were detected during the 33-month study period.

Studies conducted in the rural area of Matlab, Bangladesh (Black et al. 1982) and in Pacatuba, Brazil (Guerrant et al. 1983) yielded a similar high incidence of rotavirus infections as those detected in Cauque. A prospective study of children on Winnipeg, Canada, a population more economically developed and educated than those just mentioned, showed significantly lower rates of rotavirus than those found in Cauque or Matlab, but greater than those of Puriscal (Table 9).

The rates of rotavirus in the poor rural populations mentioned (Cauque, Matlab and Pacatuba) are below original predictions. They were, however, considerably higher than those observed in the cleaner Puriscal and Winnipeg. The rates in the first three studies are understandable on the bases of prevailing

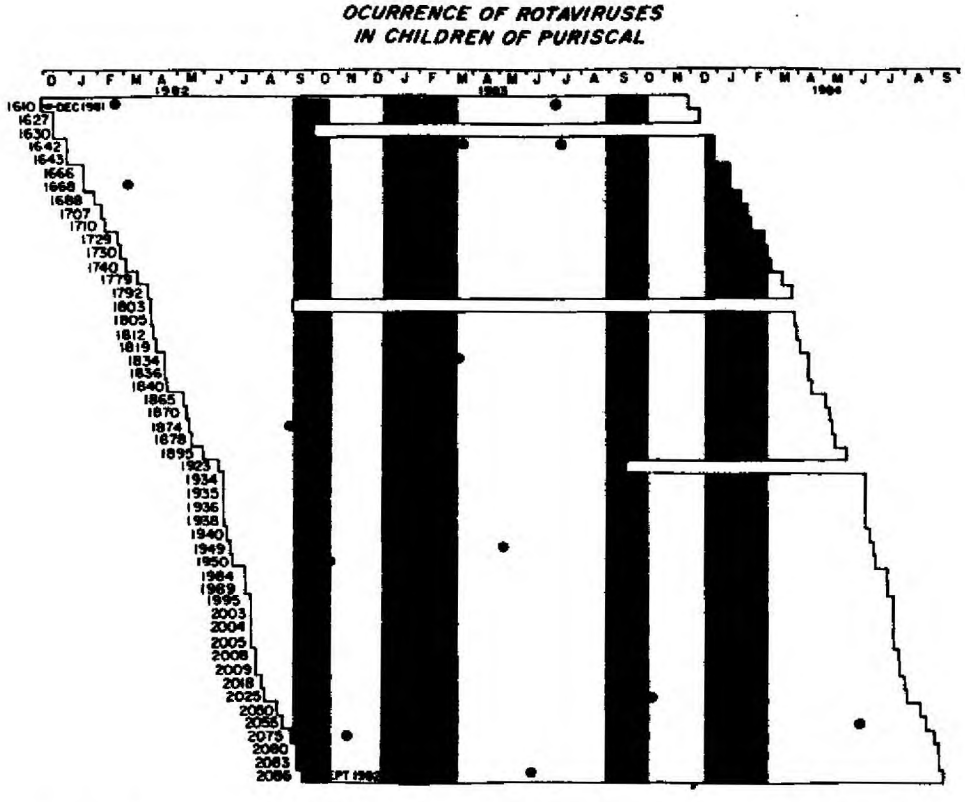


Fig. 3. Natural history of rotavirus infection in a cohort of 51 children from a Costa Rica rural region observed during the first two years of life. Identification of viruses, children and calendar are as in Fig. 2. Note that infections were significantly less than in Cauque children, while outbreaks (shaded areas) involved fewer children in Puriscal than in Cauque.
Adapted from Simhon et al. (1985).

deficient sanitation and personal hygiene. The low rate in Puriscal can only be explained by its significantly improved level of education (especially of women), better control of fecal waste and water supply, availability of health services, good level of personal hygiene, and scattering of homes in a vast area of "ruralism" (Mata, 1982b).

**TABLE 9**

**INCIDENCE OF ROTAVIRUS INFECTION AND OF DIARRHEA ASSOCIATED WITH ROTAVIRUSES IN THREE CONTRASTING ECOSYSTEMS**

| Populations | Study Parameters | | | | |
|---|---|---|---|---|---|
| | Number of Child-Years in Study | Number of Episodes (Episodes/Child-Year) of: Diarrhea, All | Rotavirus-Diarrhea | Rotavirus Infection | Pathogenicity |
| Rural, poor: | | | | | |
| Cauque | 132.5 | 1,050(7.9) | 109(0.8) | 166(1.2) | 65.7 |
| Matlab | 120 | 727(6.1) | 34(0.3) | | |
| Urban, not poor: | | | | | |
| Winnipeg | 139 | 165(1.2) | 40(0.3) | 50(0.4) | 80.0 |

Data for Matlab: Black et al. (1982).
Data for Winnipeg: Gurwith et al. (1981).
Adapted from Mata et al. (1984).

**IMPLICATIONS FOR HEALTH**

Viruses of the intestine are major causes of diarrheal disease and febrile illnesses. Such infections are a source of nitrogen loss and significant metabolic alterations with considerable negative effects on host nutrition. However, diarrheal disease and its effect on nutrition is considered the most significant public health problem in most developed countries.

Although much remains to be known, rotaviruses and adenoviruses and, to a lesser extent, the Norwalk and Norwalk-like viruses are the main contributors to winter diarrhea in temperate countries, and in the trópics as well (Wyatt and Kapikian, 1981). Rotaviruses are endemic throughout the year, with occurring epidemics during the cold season. Adenoviruses cause sporadic cases of diarrhea throughout the year.

In developing countries, rotavirus seasonality is less obvious, and yet they tend to cluster during the colder months of the year. In general, rotaviruses, adenoviruses and enteroviruses are present throughout the year in the general population, and their contribution to diarrhea is only surpassed by bacteria, namely, enterotoxigenic *Escherichia coli*, *Campylobacter* and *Shigella*. Nevertheless, rotaviruses remain as the most common agents in severe cases of diarrhea attending emergency and outpatient services in tropical countries (Linhares et al. 1983; Mata et al. 1983b), accounting for as much as 40% of all cases.

Rotaviruses replicate in the small intestine, causing a distinct lesion in enterocytes with disintegration of cell structure and derangement of the brush border (Davidson and Barnes, 1979). There is an impairment of disaccharidase function which may last for a few days or even a couple of weeks. The Norwalk agent also induces a flattening of the mucosa of the small intestine, with bridging and malabsorption similar to that observed in tropical sprue and severe malnutrition (Mata, 1983b). The immediate result of viral diarrhea is profuse loss of fluid and electrolytes, which can be so severe as to result in death by dehydration. Treatment consists of replenishing losses by prompt administration of appropriate rehydration salt solutions. The recommended approach is to rehydrate by mouth (oral rehydration therapy or ORT), except when there is severe dehydration or other dangerous signs or symptoms, for instance, persistent vomiting, shock or toxicosis. In these cases, rapid intravenous fluid therapy (RIT) is indicated and the use of appropriate antibiotics.

Rotavirus diarrhea may exhibit great severity. The clinical presentation in Cauque children was of a severe watery diarrhea with fever, vomiting, dehydration and prostration (Mata, 1983b; Wyatt et al. 1979). At the time of the Cauque study there was no knowledge or availability of ORT or RIT, and the only resource was prescription of fluid by mouth and the resource of breast feeding.

Viral diarrheas, if untreated, are a source of dehydration, nutrient wastage, nutrient losses, chronic malnutrition and death. There is anorexia and vomiting which reduce food intake or induce food wastage; there is malabsorption and impaired secretion, digestion and absorption (Molla et al. 1983a, b); and there are metabolic alterations directly related to the infectious process (Mata, 1983b).

To illustrate, the natural history of growth and diarrhea in two Cauque children is depicted in Fig. 4. These children were observed, as the rest of the cohort, in their ecosystem at a time there was not ORT or adequate medical services. Children stayed at the breast, however, for at least 18 months, and often longer, which increases survival under adverse conditions. One child was born with fetal growth retardation and the other with adequate birth weight. In both children, all diarrheas were found associated with some degree of arrest in linear growth. Previously, diarrhea and other infectious diseases had been shown related to weight loss of varying magnitude (Mata, 1983b). The effect on the growth curve tended to be more accentuated as children grew older and were less protected by the good nutrition conferred by breast feeding during the first semester of life.

There is not enough information on the impact of fastidious adenoviruses and other viruses on the nutritional status, but it is expected to be important in less developed countries. Coronaviruses had been found associated with chronic

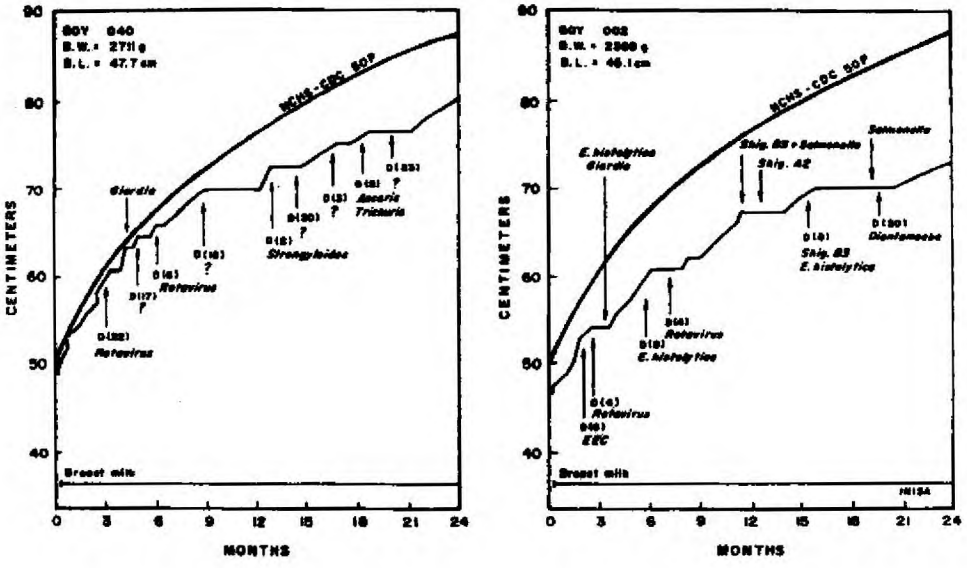


Fig. 4. Physical growth (body length, in centimeters) of two Cauque children, from birth to two years of age, compared with the 50th percentile curve of the National Center for Health Statistics (1974). Only diarrhea episodes and their etiologic associations are shown; the children suffered from a variety of other infectious diseases as well. The study was conducted at a time when there was no knowledge or technology to investigate enterotoxigenic Escherischia coli, Campylobacter, Cryptosporidium or rotaviruses. These, however, were investigated retrospectively in frozen stool extracts. Note that most diarrheal episodes were associated with periods of arrest in body growth of varying magnitude. Growth faltering was more intense after commencement of weaning. The stunting effect was greater in the child who was born with fetal growth retardation (child at right in the figure). About 30% infants experienced fetal growth retardation in the village.

From Mata (1978).

diarrhea in India (Mathan et al. 1975), and they may be contributing to malnutrition in certain deprived populations.

Diarrheas undoubtedly are the first or second contributors to childhood death in the majority of less developed countries, especially before the advent of ORT. While no comprehensive pathologic data exists to determine the contribution of viral diarrheas to mortality, extrapolation from a field study indicates that perhaps 25% of all diarrhea deaths in very poor countries may be linked to viruses (Black et al. 1982). In transitional societies, rotaviruses are proportionally more common than bacteria as a cause of diarrhea, and they may be relatively more important as a cause of death.

Viral diarrheas have among their social determinants poverty, ignorance and deprivation. While advanced medical knowledge, ORT, chemotherapy, novel treatment schemes and eventually vaccines are of unquestionable value, the ultimate control and prevention reside in improved living conditions and the quality of life.

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**DISCUSSION**

de la Maza (University of California Irvine Medical Center, Orange):

In the last two or three slides, you showed that when you were able to isolate rotavirus, the individual had a problem from the point of view of growth. There was stunted growth. The question I have is, in those individuals that you isolated rotavirus and there was stunted growth, did they have diarrhea or not? Do you have to have diarrhea in order to have stunted growth, or can you just have shedding of rotavirus and have stunted growth?

Mata L:

There is a possibility that viruses themselves, without associated clinical manifestations, can cause stunting. It is a very good possibility. A beautiful model, described by Rene Dubos at the Rockerfeller University a long time ago, where he actually found a transmissible agent, he called it a virus, in the stools of the mother mouse. When he prepared a filtrate of the stools, and put a tiny drop of this filtratable agent in the mouth of the litter at birth, it caused stunted growth. If the mice were infected when they were older, they did not become stunted. This infection with the virus was not associated with clinical manifestations, but of course, it's difficult to ask a mouse what he

feels. There was no increase in temperature, no symptoms, no changes in the hair. The only change was in body weight. So this led us, a long time ago, to try to find the same sort of thing in children of Guatemala. We never found asymptomatic infections associated with weight loss or stunting. The children that I showed in the slides were clinical diarrheal disease associated with rotavirus, Shigella or other agents, and all had an impact on the weight curve, and also on the height curve.

Fayram S (University of California Irvine Medical Center, Orange):

Dr. Mata, I noticed in your graphs that the peak incidence of rotavirus varies from year to year. One year it was January, another year it was September-October. In those years where your incidence varies, did you have a variation in your rainy season as well?

Mata L:

I wish I knew that. We kept a rain meter in the Guatemalan village. We also took the morning and evening temperatures. I didn't see anything clear. But there are so many other factors, for instance, the contamination of the water with the rotaviruses. This was the main problem of the village then, and still is the main problem. There are 5,000 villages like this in Guatemala. This is a problem of development. Other factors such as crowding of people for religious festivities, market, changes in the agricultural pattern and selling of produce, crowding of people in the home, all of these factors were not analyzed. We can say that rotaviruses are prevalent year-round in the tropics. This is being confirmed in Costa Rica, Guatemala, Venezuela, Bangladesh and Egypt. But there is an exacerbation of infection coinciding with the cooler months in these countries, too, with exceptional months with epidemics.

Purtilo D (University of Nebraska, Omaha):

Dr. Mata, you've presented a really beautiful story here. About 13 or 14 years ago, I talked the army into sending me down to Masiole, Brazil, and we looked at some children with malnutrition. We didn't have the elegant studies that you did, but we quantitated the parasite burden by counting the number of eggs and larvae and so forth, and we were just amazed at the multitude of infections in one child. The Brazilians, in trying to cope with this, had nutritional replenishment centers where they tried to educate people about that. What sort of things were being done to try to turn this around where you've worked and where you live now?

Mata L:

All of these issues are bound to be influenced by emotional attitudes, which is the most dangerous thing that can happen to the country, to the government and to scientists. Also, they are influenced by profound personal convictions. Regarding the approach, there have been two schools, and I have always been in the minority. Because I am a microbiologist primarily, this has probably influenced my way of seeing facts. When I worked in Guatemala, I didn't think that the problem was food. Why? Because, myself, I haven't eaten so much in my life. I ate mainly rice, beans, bread and tortillas. I rarely drank milk, cheese or meat, and I found that I was healthy and actually tall. No taller than many Americans, 5 feet 8 inches, but tall for village standards. I found these two other guys in the village who were taller than me, and certainly twice as strong, and were raised exclusively with rice and beans and with some leaves. They had gone through everything, dysentery, typhoid, hepatitis, malnutrition. So I proposed the hypothesis that it was not lack of food, but the burden of infection which made the people malnourished. The United States offered us money, one million dollars to do two studies. One was to make the diet perfect, which is what everybody wanted, because eating is so important, particularly for your society. The other grant was to teach families, especially women, to wash their hands and to improve their hygiene, and although it was not an appealing proposal, there was also a half a million dollars to change the community in this respect. I consulted with my boss, and my boss said, if you want to be a really good scientist, you have to follow Pasteur's approach. You have to use the hypothesis that you don't like, and try to prove that you are wrong. So I picked up the half a million dollars to do the nutrition study, and we left Dr. Schneider, a gastroenterologist, to do the other study. We got from the United States soy flour that contained synthetic lysine and all other nutrients, including iron, in an available source that would become absorbed. By adding this to the local corn dough for tortilla, which is the staple food in that area, it would have nutritional requirements comparable to meat. For four years, we fed the villagers with this food. One problem was that many people did not like it. We put a marker on the tortilla (riboflavin) and then sampled the urine of women to make sure that they were eating it. After four years, we proved that there was no change whatsoever in any parameter, including birth weight, morbidity, or mortality. It was a very frustrating experience. The other researcher showed that, if you teach women how to wash their hands and other hygienic measures, you could cut down the rate of diarrheal disease in half or by a third. However, he didn't examine if that effect of the hand washing and cleaning the child had an effect on their nutrition. So we were left, more or less, with a negative experiment. A positive experience, but no definite answer on this. I went to Costa Rica

thinking that there was a lot of malnutrition. So we analyzed the data on mortality in Costa Rica, and with surprise, we found that my country had an infant mortality of 30, almost the same as the Soviet Union, one of the most advanced nations in the world. We thought there must be something wrong. If the mortality is so low, Costa Rica must have a low rate of malnutrition. So we did an analysis of a recent survey and found that malnutrition had declined in Costa Rica. As a matter of fact, at this moment, the rate of malnutrition in the village that I discussed is less than that in Massachusetts. One possible explanation was that people were eating more. But when we studied the diets of women and children, we were surprised to know that, actually, the women in Guatemala were eating more protein and more calories than those Costa Rica. However, Costa Rican women were heavier, and the children were growing better than in Guatemala. So we thought that there must be another explanation. The only explanation that we have is the marked decline in infectious diseases, which are now very low, particularly diarrheal diseases. This was due to cleaner water, more education, and better hygiene. Costa Rica consumes more toothpaste, more sanitary napkins and toilet paper than the rest of Central America together, excepting Panama. And Panama is also like Costa Rica, more advanced. This is a good index of personal hygiene. In summary, if you are clean, than you probably eat more of what you have around, and whatever you eat will be utilized better.

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# PAPOVAVIRUSES: BIOLOGY, PATHOGENIC POTENTIAL AND DIAGNOSIS

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## INTRODUCTION

Until the mid 1960s, no serious human illness was related to infections with human papovaviruses, although it was known that human warts were caused by one or more viruses of this family. The term papovavirus is an acronym derived by grouping together papillomaviruses of man and rabbit, polyomavirus of mouse and vacuolating virus (SV40) of rhesus macaque (Melnick, 1962). These viruses share the properties of small size, naked icosahedral capsid, double-stranded circular DNA genome and nuclear multiplication. Subsequent studies have shown that the papovavirus family consists of two unrelated genera, papillomavirus and polyomavirus, which differ in many respects (Table 1). Papillomaviruses are larger than polyomaviruses (55 nm vs. 45 nm diameter) and carry their genetic information on one strand. Polyomaviruses carry about equal information on each of the two strands. Papillomaviruses infect epithelial surfaces and produce their pathology at the site of entry. Polyomaviruses enter the body by the respiratory or other routes and reach their target organs by viremia. The virus groups also differ in their ability to produce tumors. The typical outcome of a naturally occurring papillomavirus infection is cellular proliferation and the production of a benign self-limiting tumor. All of the cells of the papilloma contain the viral genome which is present as extrachromosomal, free, unintegrated copies. However, permissive infection leading to the synthesis of viral particles occurs only in cells of the upper layers of the epithelium which are differentiated and are destined to die (Noyes and Mellors, 1957). A papillomavirus lesion may progress towards malignancy. This process is accompanied by inhibition of synthesis of viral capsid proteins and, in many cases of invasive cancers, by the integration of the viral genome in the cellular DNA. Papillomaviruses are associated with naturally occurring cancers in many species, including man. In contrast, polyomaviruses have not been convincingly implicated in naturally occurring tumors in any species. They are cytopathic in their natural hosts. Infection leads to synthesis of virus particles and lysis of infected cells. However, in experimental systems, polyomaviruses are highly oncogenic for laboratory animals and are capable of transforming cells from a wide variety of species.

All papillomaviruses share some conserved nucleotide sequences and antigenic determinants, attesting to their common evolutionary origin (Heilman et al. 1980; Jenson et al. 1980). Similarly, all polyomaviruses can be shown to be inter-