Summer Outbreaks of Influenza

To the Editor—Kohn et al. [1] recently described three sum­mer time outbreaks of influenza type A. How the influenza virus is perpetuated during the summer is intriguing. A prevailing hypothesis is that influenza viruses are carried to colder climates at the end of the season to return with travelers the next season. However, while influenza is infrequently isolated in summer months, it does not completely cease to circulate during interepidemic periods [2], suggesting there is some mode of viral persistence in the population after an epidemic. Latent virus could persist in individuals until reactivated a year or more later [3] or virus may survive between epidemics by sequential transmission from person to person at levels below the epidemic threshold [4].

Experiments in ferrets support the latter hypothesis. Groups of 4 ferrets were inoculated intranasally as described previously [5] with 10⁶, 10⁴, or 10² 50% EID₅₀ of clone 7a (H3N2) of A/Puerto Rico/8/34-A/England/939/69 reassortant virus under ether anesthesia. Virus and inflammatory cell levels in nasal washes were monitored and pyrexial temperatures were recorded for up to 7 days after infection [5]. In animals inoculated with 10⁶ EID₅₀, virus peaked at 30 h (10⁴.950% egg bit infectious doses [EBID₅₀]/mL) and declined to barely detectable levels by 78 h after infection (figure 1A). In contrast, virus shedding from animals inoculated with 10⁴ EID₅₀ peaked a little lower (10⁴.3 EBID₅₀/mL) and later (48 h), becoming undetectable by 78 h after infection. Peak titers in animals inoculated with 10² EID₅₀ were delayed until 54 h after infection, reduced (10² EBID₅₀/mL), and not detectable by 78 h after infection. Using the virus index, a mathematically calculated area under the curves of individual animals [6], animals inoculated with 10² EID₅₀ shed significantly (P < .001) less virus (mean ± SE = 50.8 ± 2.1) than did animals inoculated with either 10⁴ (73.4 ± 4.7) or 10⁶ (89.0 ± 5.1) EID₅₀. Although the difference was not statistically significant (P > .05), virus levels with the 10⁴ inoculum were also less than with 10⁶ EID₅₀.

Similarly, the nasal inflammatory response, indicative of the severity of respiratory symptoms, was considerably higher with the 10⁶ inoculum (mean total cells = 10⁷.5) than with the 10⁴ (10⁶.6 cells) or 10² (10⁶.2 cells) inocula (figure 1B). Again according to the cell index [6], animals given 10⁷ EID₅₀ produced significantly fewer (P < .001; mean ± SE = 45.1 ± 2.1) nasal cells than did animals inoculated with either 10⁴ (80.3 ± 2.9) or 10⁶ (143.1 ± 6.4) EID₅₀, and animals inoculated with 10⁵ EID₅₀ produced significantly fewer cells (P < .001) than did animals inoculated with 10⁶ EID₅₀.

Animals inoculated with 10⁶ EID₅₀ were febrile by 30 h after infection; fever lasted ~30 h (range, 30–60; peak mean temperature rise, 2.9°C; figure 1C). In animals inoculated with 10⁴ EID₅₀, fever occurred later (36 h after infection) and had a lower peak (2.0°C) and shorter duration (24 h). In animals inoculated with 10² EID₅₀ (figure 1C), fever was further reduced to a peak of 1.8°C with a 12-h duration. The mean fever indices [6] were 42.5 ± 8.0, 28.9 ± 6.2, and 21.1 ± 3.5°C for the 10⁶, 10⁴, and 10² EID₅₀ inocula, respectively; the fever index for
the $10^2$ EID$_{50}$ was significantly lower ($P < .05$) than for the $10^6$ inoculum.

Low inocula therefore produce significantly less virus than higher inocula and are associated with less respiratory (nasal temperatures and relative humidity) may reduce inocula, leading to low-level shedding with expression of asymptomatic or "cold-like" rather than "influenza-like" symptoms [2]. Transmission would be reduced because of poor replication and reduced nasal discharge. However, during the winter season, environmental conditions (reduced relative humidity and air temperature, impaired ciliary action following chilling of the respiratory tract, overcrowding, and increased sneezing and coughing) facilitate enhanced replication and aerial transmission of the virus [7]. Initially, virus levels may be sufficient only to infect children who are more susceptible than adults and usually represent the index case [8]. Children shed more virus for longer periods than adults [9], increasing the level and duration of transmitted virus and ultimately leading to symptomatic infection in adults. Such low-level transmission may allow new variants to emerge at different times during the summer season, depending upon prevailing climatic conditions [1]. Monitoring for influenza in the summer in temperate climates may indicate viruses likely to cause epidemics or pandemics in the next winter season.

Pathogenic Escherichia coli in Children with and without Chronic Diarrhea in Tanzania

To the Editor—We read with great interest the article by Mathews et al. [1] in which the frequencies of isolation of HEp-2 cell-adherent Escherichia coli from human immunodeficiency virus (HIV)—positive and -negative adults with diarrhea were compared. Their subjects were presented to a university hospital in Lusaka, Zambia. Mathewson et al. found significantly more HEp-2 cell-adherent E. coli in HIV-positive than in HIV-negative adults with chronic diarrhea (30/38 vs. 1/6, $P < .002$). We have extensively analyzed pathogenic E. coli in children with chronic diarrhea in Dar es Salaam, Tanzania, and used HEp-2 cell adherence and DNA probes for seven types of diarrheagenic E. coli. Our results differ from those of Mathewson et al. Thus, we caution that their findings may not generalize to other geographic locations and age groups.

Our study was done at Muhimbili Medical Center, a 1500-bed urban academic center hospital. Eligible subjects and controls were children 15 months to 5 years of age who were admitted from August 1989 to January 1990. Subjects had chronic diarrhea; controls were hospitalized for acute trauma and did not have diarrhea. Each participant had a standardized clinical evaluation that included stool studies and HIV serology. The lower age limit was chosen in order to exclude children with false-positive HIV serology due to maternal, transplacentally acquired anti-HIV antibody [2, 3]. Diarrhea was defined as three or more unusually loose bowel movements per day. Chronic was defined as diarrhea persisting $\geq 2$ weeks in the preceding month. Two fresh stool specimens collected in the first 2 days of hospitalization were frozen at $-70^\circ C$, transported to the United States in liquid nitrogen, and inoculated onto MacConkey agar and into gram-negative broth for enrichment and then onto MacConkey agar. Three colonies of E. coli were picked from each specimen and grown on trypticase soy agar for DNA probing and HEp-2 cell adherence assays.

The derivation of the DNA probes and the techniques used for DNA probing have been described [4]. In brief, colonies of each isolate were transferred onto nitrocellulose filters and dried. The DNA was denatured and hybridized with eight different $^{32}$P-labeled DNA probes for seven types of E. coli: enteropathogenic (EPEC), diffuse adherence (DAEC), enterotoxigenic of both heat-labile and heat-stable toxin types, enteropathogenic (EPEC; EPEC-adherence factor [EAF] and EPEC-attaching and effacing locus), enteroinvasive (EIEC), and enterohemorrhagic (EHEC). Hybridization to the colony blots was detected autoradiographically. The HEp-2 cell adherence assay for EAggEC, DAEC, and EPEC (localized adherence) has been described [4]. Monolayers of HEp-2 cells were grown on glass coverslips in 24-well tissue culture plates. Wells were inoculated with 20 $\mu$L of broth bacterial culture with

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References


Patients or guardians gave informed consent. The study was approved by the Ethics Committee, Muhimbili Medical Center.

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