SUPPRESSION OF PLASMODIUM FALCIPARUM INFECTIONS DURING CONCOMITANT MEASLES OR INFLUENZA BUT NOT DURING PERTUSSIS

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Abstract. In tropical countries, concomitant infections are a continuous problem. In the Rufiji Delta, an area of Tanzania that is holoendemic for malaria, there were outbreaks of influenza A, measles, and pertussis in 1986 and 1987. Significantly lower parasitic prevalences and mean densities of malaria parasites were found in children up to nine years of age who had measles or influenza than in asymptomatic control children. In contrast, children with pertussis had a higher prevalence and mean density than controls. The clinical courses of measles, influenza, or pertussis infections did not appear to be significantly affected by concomitant malaria infections. The reasons for the suppression of Plasmodium falciparum parasitemia during these viral infections are unclear. This effect could not be explained by the presence of fever.

In tropical countries, concomitant infections are a continuous problem. The general view is that an acute nonmalarial infection in an otherwise asymptomatic Plasmodium falciparum carrier will be associated with a transient increase in the number of parasites and possible exacerbation of malaria symptoms. Thus, in holoendemic areas, episodes of fever are often thought to involve either exacerbations of chronic malaria infection due to concomitant viral infections or superinfections with parasites to which the host has less immunity. This results in a widespread use of antimalarial drugs that enhances the alarming development of P. falciparum resistance to the drugs used.

In the Rufiji Delta in eastern Tanzania, an outbreak of influenza occurred in September-October 1986, one of measles during the spring of 1987, and one of pertussis in October 1987. We studied the possible interactions in patients between these three diseases and concomitant P. falciparum infection during these three epidemics.

PATIENTS AND METHODS

Study area

The village of Nyamisati is situated at latitude 7°S in the Rufiji River delta, near the Indian Ocean in eastern Tanzania. The population is of Bantu origin with an Arabic influence. The main occupations are fishing and agriculture (rice cultivation). A Primary Health Care Clinic had recently been established and was run by our research team in the village. Medical treatment was in practice available only through the clinic. The main diseases of the villagers are malaria, gastroenteritis, upper respiratory tract infections, pneumonia, and lymphatic filariasis. The nutritional status is normally adequate because of continuous access to fish.

Between 1986 and 1988, a longitudinal clinical study of malaria was conducted in the village. Malaria was found to be holoendemic; transmission was perennial with some seasonal fluctuations. All fever episodes were systematically investigated and treated according to a diagnosis based on the clinical history, physical examination, and basic laboratory tests.

Influenza epidemic

An epidemic of influenza-like symptoms occurred in Nyamisati in September and October 1986. The influenza diagnosis was based on upper respiratory tract symptoms, fever and/or headache, and general body pain. Blood films for the detection of malaria parasites were made from each patient at the time of acute influenza infection. Routine treatment of the influenza symptoms included antipyretics and analgesics. All patients were followed for additional clinical manifestations for one month after the diagnosis.
of influenza to establish that the clinical course was normal. In 24 patients, blood samples were again collected 3–4 weeks after the acute stage for serologic confirmation of the diagnosis of influenza. The sera were centrifuged, stored at −18°C, and compared with sera that were obtained in a survey six months before the epidemic.

Measles epidemic

An outbreak of measles occurred in Nyamisati and surrounding villages between March and May 1987, despite the fact that a measles vaccination campaign had supposedly been conducted in recent years in this area by mobile medical units. A measles diagnosis was based on fever with typical rash and photophobia. Routine blood films were simultaneously made from each patient for the detection of malaria parasites. The patients were given Vitamin A (50,000 units) and antipyretics as recommended by the Tanzanian Ministry of Health. They were told to return to the clinic immediately if clinical manifestations of secondary infection or aggravation of measles occurred. All patients were followed up to three months after the measles diagnosis to confirm clinical improvement.

An additional blood film was routinely made for 32 patients from Nyamisati 2–4 weeks after the acute clinical stage of measles. Hematocrit tubes of blood were also obtained, centrifuged, and the plasma samples were stored at −18°C for serologic confirmation of the measles diagnosis. Unfortunately, 20 samples spoiled during transport to Sweden.

Pertussis epidemic

An outbreak of pertussis occurred in Nyamisati village in September and October 1987, although as with measles, vaccination against pertussis had supposedly been performed in the area in recent years by a mobile medical clinic. A pertussis diagnosis was based on clinical symptoms of gasping cough (whooping); fever was not an obligate symptom. Routine blood films were made simultaneously for each patient for the detection of malaria parasites. All children were followed for three months to confirm clinical improvement. Plasma was collected from 19 children 2–4 weeks after the acute clinical stage. The samples were centrifuged, and stored at −18°C for detection of pertussis antibodies. These samples were compared with samples obtained from the previous routine survey in the village.

Controls

Asymptomatic individuals were selected as controls when patients in each epidemic were identified. Blood films were obtained at the same time and similarly examined for malaria parasites. For each patient in the two viral epidemics, an age-matched control was selected from nearby houses in the same village. Controls for the pertussis epidemic consisted of 231 children up to the age of 14 years identified in the general survey performed at the same time in the entire village. Weighted parasite prevalences and means were calculated from these individuals.

Laboratory methods

Microscopic diagnosis of malaria was done on a thick blood film stained with Giemsa. Parasite densities were calculated for leukocytes, assuming a leukocyte count of 8,000/μl, except for the pertussis patients, in whom the leukocyte count was assumed to be 20,000/μl since they were not previously immunized. A blood film was considered negative if no parasites were detected in 100 microscopic fields using a magnification of 1,000 x.

The serologic tests for the confirmation of viral and bacterial infections were done at the National Bacteriological Laboratories (Stockholm, Sweden). Complement fixation tests were performed for influenza A and B group antigens according to the well-known method of Takatsy. A positive response at a dilution ≥ 1:80 or a four-fold increase in the titer of paired samples was considered to be indicative of influenza infection.

An enzyme-linked immunosorbent assay (ELISA) was used to detect measles IgM antibodies. The assay was performed using microplates coated with rabbit anti-human IgM diluted to 1:1,000. An absorbance value > 0.2 was considered positive. The ELISA was also used to detect IgG and IgA antibodies to the pertussis filamentous hemagglutinins (FHA) and IgG antibodies to the lymphocytosis promoting factor (LPF). A two-fold increase in FHA-IgG, FHA-IgA, and LPF-IgG titers in paired serum samples or a two-fold increase in titers above controls (controls based on average titers from 50 healthy
Parasite densities (mean log$_{10}$n + 1) and prevalences (% positive) of patent parasitemia in patients with influenza and age-matched controls from a malaria holoendemic village in Tanzania*  

<table>
<thead>
<tr>
<th>Age (years)</th>
<th>n</th>
<th>Influenza</th>
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<th>Controls</th>
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<tr>
<td></td>
<td>n</td>
<td>Parasites/µl</td>
<td>% Positive</td>
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<td>% Positive</td>
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<td>geometric mean (range)</td>
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<td>geometric mean (range)</td>
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<td>18</td>
<td>20 (0–5.680)</td>
<td>67</td>
<td>84 (0–11.120)</td>
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<td>12</td>
<td>8 (0–2.320)</td>
<td>50</td>
<td>73 (0–8.000)</td>
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<td>15</td>
<td>36 (0–2.560)</td>
<td>67</td>
<td>64 (0–4.480)</td>
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<tr>
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<td>16 (0–1.120)</td>
<td>64</td>
<td>4 (0–1.200)</td>
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<tr>
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<td>9 (0–320)</td>
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<td>8 (0–880)</td>
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<tr>
<td>11</td>
<td>6 (0–2.560)</td>
<td>64</td>
<td>2 (0–16)</td>
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<tr>
<td>21</td>
<td>3 (0–240)</td>
<td>43</td>
<td>7 (0–2.560)</td>
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<td>1 (0–32)</td>
<td>17</td>
<td>3 (0–240)</td>
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* For parasite density, P = 0.01 for 0–9-year-old children versus controls, P = 0.09 for 10–14-year-old children versus controls, and P = 0.8 for 14–30-year-old individuals versus controls (by paired t-test). For prevalence, P = 0.02 for 0–9-year-old children versus controls (by dependent chi-square test).

Infants (0–30-year-old age group, 12 (31%) of 39 patients with influenza-like disease had patent parasitemia ranging from 8 to 240 parasites/µl, whereas in the control group, 19 (49%) of 39 had parasitemia ranging from 8 to 2,560 parasites/µl. Again, no significant difference was found in the parasite prevalences or densities.

RESULTS

Influenza epidemic

A total of 128 patients were clinically diagnosed as having influenza, and all age groups were represented (Table 1). Three patients developed a concomitant pneumonia, but were successfully treated with antibiotics. All the remaining patients recovered from the clinical episode and all were in good health during the follow-up period of one month. A serologic verification of the diagnosis of influenza A was made in 14 (58%) of 24 patients tested. None had increased titers against influenza B.

The observed parasite prevalences and densities for malaria in the patients with influenza symptoms were compared with those of the controls (Table 1). In the 0–9-year-old children, 28 (62%) of 45 had patent parasitemia, ranging from 8 to 5,680 parasites/µl. In the control group of asymptomatic children, 38 (84%) of 45 had parasitemia ranging from 8 to 11,120 parasites/µl. The control group had a significantly higher parasite prevalence (P = 0.02) (Figure 1) and a mean positive parasite density (P = 0.01) (Figure 2). In the 10–29-year-old patients with influenza, 27 (61%) of 44 had parasitemia ranging from 8 to 240 parasites/µl, whereas in the corresponding control group, 19 (43%) of 44 had parasitemia ranging from 8 to 1,200 parasites/µl. There was no statistically significant difference in parasite prevalence or density between the influenza patients and the controls in this age group. In the > 30-year-old age group, 12 (31%) of 39 patients with influenza-like disease had patent parasitemia ranging from 8 to 240 parasites/µl, whereas in the control group, 19 (49%) of 39 had parasitemia ranging from 8 to 2,560 parasites/µl. Again, no significant difference was found in the parasite prevalences or densities.

Measles epidemic

A total of 67 patients were diagnosed as having measles. The clinical symptoms of these children were all classic, with photophobia and rash. There

![Graph](image-url)
In the control group of asymptomatic subjects, 59 (88%) of 67 had parasitemia ranging from 8 to 34,400 parasites/μl. Both the prevalence and the parasite density were significantly lower in the measles patients than in the controls ($P < 0.001$) (Figures 1 and 2).

From days 14 to 35 after the acute stage of measles, 28 patients were again examined for malaria parasites. Nineteen (68%) had detectable parasitemia ranging from 160 to 5,200 parasites/μl, with a geometric mean of 222 parasites/μl. These findings more closely resembled those previously reported in the control children and in individuals surveyed before the measles epidemic (unpublished data).

### Pertussis epidemic

A total of 24 children were diagnosed as having clinically typical pertussis. The clinical course of the disease was as expected, with continuing cough for up to eight weeks. There were no deaths, and all patients recovered within this period of time. One child needed treatment for a concomitant pneumonia. Serologic verification of the diagnosis was made in 18 of 19 sera tested. Of the 16 children with paired serum samples, 15 had significant increases in FHA-IgG titers and 14 had significant increases in LPF-IgG titers. The two children with only a single serum sample both had high FHA-IgG titers. No child had been previously vaccinated against pertussis.

The observed malaria parasite densities in the pertussis patients at the time of the diagnosis are presented in Table 3. One child less than three months old was not included in the study on concomitant malaria infection because of assumed protection by passively transferred maternal antibodies. Of the remaining 23 children...
with pertussis, 20 (87%) had parasitemias ranging from 8 to 13,000 parasites/μl, and in the control group of asymptomatic children, 141 (61%) of 231 had parasitemias ranging from 8 to 8,200 parasites/μl (Figures 1 and 2). Whereas the difference in prevalence was not statistically significant (P = 0.33), the mean parasite density in the pertussis-infected children was significantly higher than in the controls (P < 0.05).

Fever

All children less than ten years old with influenza reported fever. In seven of nine children examined with a thermometer at the clinic, a confirmed axillary temperature of > 38.0°C was observed. The mothers of the measles patients reported fever in 25 of 27 children from Nyamisati, and 15 of 27 had an axillary temperature > 38.0°C, as measured at the clinic. Mothers reported fever in three of 13 pertussis-infected children, and all had a confirmed axillary temperature > 38.0°C. One of the children with a fever had a concomitant pneumonia. In all three epidemics, only children whose mothers reported fever did have confirmed temperatures > 38.0°C.

Discussion

In tropical countries, children often have several concomitant infections, and interactions between infections therefore represent an important clinical and diagnostic problem. We have studied the interactions of influenza, acute measles, and pertussis with malaria, and have specifically investigated the possible exacerbation of clinical malaria infection at the time of these acute bacterial and viral infections.

Among the influenza patients, 14 of 24 serologically tested patients had titers considered indicative of influenza A, thus confirming the existence of the epidemic. The serologically unverified diagnosis in the other patients may suggest another diagnosis, but all of them had similar upper respiratory tract symptoms with fever and/or general body pain, which supports a similar viral etiology. All children clinically diagnosed as measles who were tested serologically during the convalescent period had positive IgM titers. This confirms the existence of the measles epidemic, and also makes this diagnosis most likely in the clinically diagnosed patients who were not tested for IgM antibodies. The fact that only two of 67 patients diagnosed with measles reported previous vaccination indicates that vaccine coverage was insufficient in this area, and suggests that this, rather than inadequate vaccine quality, was the reason for the epidemic. In the pertussis epidemic, 18 of 19 patients tested had serologically verified infections.

In the two viral epidemics, we found significantly lower parasite prevalences and densities in patients less than 10 years old compared with control children (Tables 1 and 2 and Figure 1). We are aware that the leukocyte count is often reduced during acute viral infection, and that the assumed count of 20,000 leukocytes/μl may be too high, but this would make the results even more significant. Unrecorded intake of antimalarial drugs cannot explain the low parasitemias, since drugs are only available through the clinic. In contrast to the children with viral infections, parasitemias in the pertussis patients were even higher than in the controls. Again, the difference may be underestimated because the assumed count of 20,000 leukocytes/μl is probably an underestimate (Figure 2).

Other infections are considered to cause a flareup of clinical malaria because of associated immunosuppression. This was not confirmed in our study. To our knowledge, there is no reported

### Table 3

<table>
<thead>
<tr>
<th>Age (years)</th>
<th>Parasites/μl, geometric mean (range)</th>
<th>% Positive</th>
<th>Controls</th>
<th>Parasites/μl, geometric mean (range)</th>
<th>% Positive</th>
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<td>0-5</td>
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<td>49 (0–2,200)</td>
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<td>74</td>
<td>63 (0–4,600)</td>
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</tr>
<tr>
<td>0-14</td>
<td>145 (0–3,800)</td>
<td>63</td>
<td>18 (0–800)</td>
<td>63</td>
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</tr>
</tbody>
</table>

* For parasite density, P < 0.05 for 0-9-year-old children versus controls (by non-paired t-test). For prevalence, P = 0.33 for 0-9-year-old children versus controls (by non-dependent chi-square test).
study in humans on the effect of viral or bacterial infections on malaria. However, under experimental conditions in mice, exacerbations of malaria by infections of other agents, including other protozoa and different lymphogenic viruses, have been observed. Synergistic interactions have also been found between *P. yoelii* and helminths in concurrent experimental infections.

A general lymphocytic malfunction has been observed in measles and influenza, suggesting immunosuppression by the viral infection. However, although splenectomy increases the severity of malaria and immunosuppressive therapy after renal transplantation also appears to have this effect, we believe that viral infections do not significantly reduce immunity against *P. falciparum* in humans, but seem to facilitate clearance of these parasites.

The reason for the observed decrease or clearance of parasitemia during the viral infections is not known; however, several mechanisms are possible. Fever is one such potential factor. It is known that intraerythrocytic parasites, which survive in culture at 37°C, are damaged by exposure to a temperature of 40°C during the second half of the 48-hr growth cycle. High fever was reported in nearly all children with the two viral infections. However, in the same study area, 0-9-year-old children with bronchopneumonia and high fever had mean parasite densities that were similar to that of the control group (Rooth I. and others. unpublished data).

Measles and influenza are systemic infections, and the presence of virus in the blood may possibly cause nonoptimal conditions for parasite growth. In contrast, pertussis is more localized in the trachea and bronchi. Low zinc and low iron levels seen during an acute illness of viral etiology may also be considered as causes of parasite suppression. However, a rapid decrease in plasma iron and zinc concentrations was also seen in patients with acute infections of bacterial origin, such as the children with pneumonia, who did not show suppressed parasitemia. Generally, competition for nutrients may, however, add to the complexity of heterologous interactions.

The roles of different cytokines have been recently discussed in connection with immunity to malaria. Levels of interferon-gamma and other cytokines have been found to be elevated during measles infection. Interferon-gamma levels were increased primarily during the early rash stage of the disease.

Nyamisati is holoendemic for malaria and the children living there are sporadically parasitemic throughout the year, with an average point prevalence of 86% (unpublished data). In our study, clinical manifestations in the measles patients were not more severe or milder than expected, and in the pertussis patients the symptoms were apparently not aggravated by concomitant *P. falciparum* parasitemia.

Malaria infection is known to be immunosuppressive. General immunosuppression may possibly affect the clinical manifestations and outcome of concomitant viral or bacterial infections. Indeed, enhanced infections and more severe disease have been induced by various microbial agents in malaria-suppressed mice. However, chronic malaria does not appear to influence morbidity or mortality of gastroenteritis or respiratory tract infections.

The microscopic detection of parasites in children with fever from a holoendemic area does not necessarily imply that malaria infection is the cause of fever. However, our findings in Nyamisati indicate that malaria parasite densities during viral infections are reduced rather than increased, and are consequently much lower than parasite densities in the same population during clinical malaria episodes (unpublished data). Estimation of parasitic density may therefore represent an important tool for etiologic diagnosis of fever and optimal patient treatment.

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