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SHORT REPORT: POSITIVE CORRELATION BETWEEN ROSETTING AND PARASITEMIA IN *PLASMODIUM FALCIPARUM* CLINICAL ISOLATES

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Abstract. *Plasmodium falciparum* isolates that form rosettes with uninfected red cells are associated with severe malaria in African children, although the mechanism by which rosetting contributes to severe disease is unknown. Here we have analyzed the relationship between rosetting and parasitemia in two samples of clinical isolates from children with malaria in Kilifi, Kenya. A consistent positive correlation was found between rosetting and parasitemia (Spearman's rank correlation coefficent $\rho = 0.467$, $P < .001$, n = 154, for 1993 study; $\rho = .407$, $P < .001$, n = 74, for 2000 study). Rosetting may enhance parasite growth and survival by facilitating invasion or promoting immune evasion, thus allowing higher parasitemia to develop and increasing the likelihood of severe malaria.

Infected erythrocytes from some *Plasmodium falciparum* isolates bind uninfected erythrocytes to form clumps of cells known as rosettes.[1] Isolates with a high level of rosetting have been associated with severe malaria in many e.g.[2,3] but not all[4] studies in Africa, whereas this disease association is not seen in Southeast Asia[5,6] or Papua New Guinea.[7] It has been suggested that rosetting may be a virulence factor for severe disease in African children, possibly because rosetting infected erythrocytes cause greater obstruction to microvascular blood flow than nonrosetting infected cells.[8]

The molecular basis of rosetting is under investigation, and a parasite ligand and several uninfected red cell receptors have been identified.[9,10] It is still unknown why parasites form rosettes, although the occurrence of rosetting in all four species of plasmodia that infect humans[11] and various plasmodia from other hosts[12] raises the possibility that rosetting could have an important role in host–parasite interactions. It has been suggested that rosetting could enhance parasite growth and survival within the human host either by facilitating invasion[1,13] or by acting as an immune evasion mechanism.[14] Rosetting could enhance invasion by allowing merozoites to move directly from a ruptured schizont into the uninfected red cells forming a rosette, without passing through the host's plasma. Careful experiments using a *P. falciparum* culture-adapted parasite clone showed no evidence for increased invasion efficiency in rosetting compared with nonrosetting parasites.[13] It remains possible, however, that such an effect would only be seen in the presence of some degree of host invasion-blocking immunity to merozoite antigens, and this has yet to be tested experimentally. The second hypothesis is that rosetting could act as a "cloaking device," so shielding the infected erythrocyte from host phagocytic cells or antibodies that could lead to clearance of infected cells by the immune system.[15] No experimental evidence exists to support or refute this hypothesis.

If rosetting either facilitates invasion or acts as an immune evasion mechanism, it can be predicted that isolates with high levels of rosetting should reach higher parasitemia in vivo than isolates with no or low rosetting. We therefore set out to study the relationship between rosetting and parasitemia in *P. falciparum* clinical isolates. Initially, however, it was necessary to address the question of whether high parasitemia itself influences the amount of rosetting seen, and this was studied using a *P. falciparum* culture-adapted clone IT/R29. A culture was diluted so that after invasion four flasks ranging in parasitemia from 0.3% to 15.5% were obtained. Rosetting was assessed by microscopy when the parasites reached the mature pigmented trophozoite stage and was found to be consistent across the four flasks (between 61% and 65% of infected erythrocytes in rosettes). This indicates that rosetting is an inherent property of a given *P. falciparum* isolate that is not directly influenced by the parasitemia of a culture.

TABLE 1

Patient details, rosetting, and parasitemia in *P. falciparum* clinical isolates

Disease category	n	Age (mo)*	Hb (g/dL)*	Parasitemia (%)*	Rosette frequency (%)†	Rosetting-parasitemia correlation‡
1993						
All isolates	154					$\rho = .467$, $P < .001$
Severe§	36	33 (20)	6.9 (2.3)	12.6 (11.4)	7 (2–26)	$\rho = .470$, $P = .005$
Moderate¶	64	18 (20)	7.0 (2.1)	8.3 (8.7)	5 (1–12)	$\rho = .589$, $P < .001$
Mild#	54	41 (27)	8.6 (2.0)	3.9 (2.8)	1 (0–6)	$\rho = .154$, $P = .263$
2000						
All isolates	74					$\rho = .407$, $P < .001$
Severe	25	27 (22)	6.6 (3.8)	12.1 (9.9)	14 (4–32)	$\rho = .542$, $P = .008$
Moderate	30	30 (21)	8.1 (2.5)	9.0 (8.4)	4 (1–10)	$\rho = .351$, $P = .058$
Mild	19	38 (22)	8.6 (1.2)	4.3 (2.6)	1 (0–7)	$\rho = -.286$, $P = .207$

Severe malaria was defined by the presence of febrile illness with *P. falciparum* parasitemia with unrousable coma or respiratory distress (abnormally deep breathing) or prostration (unable to sit, or in infants, breast-feed). Moderate malaria was defined by febrile illness with *P. falciparum* parasitemia and no features of severe disease but with additional symptoms requiring inpatient care such as anemia, hyperparasitemia, seizures, or vomiting. Mild malaria was defined by febrile illness with *P. falciparum* parasitemia and no features of severe or moderate disease. All mild cases were treated as outpatients.

* Values represent mean ± standard deviation.

† Values represent median and interquartile range.

‡ Spearman's rank correlation coefficient.

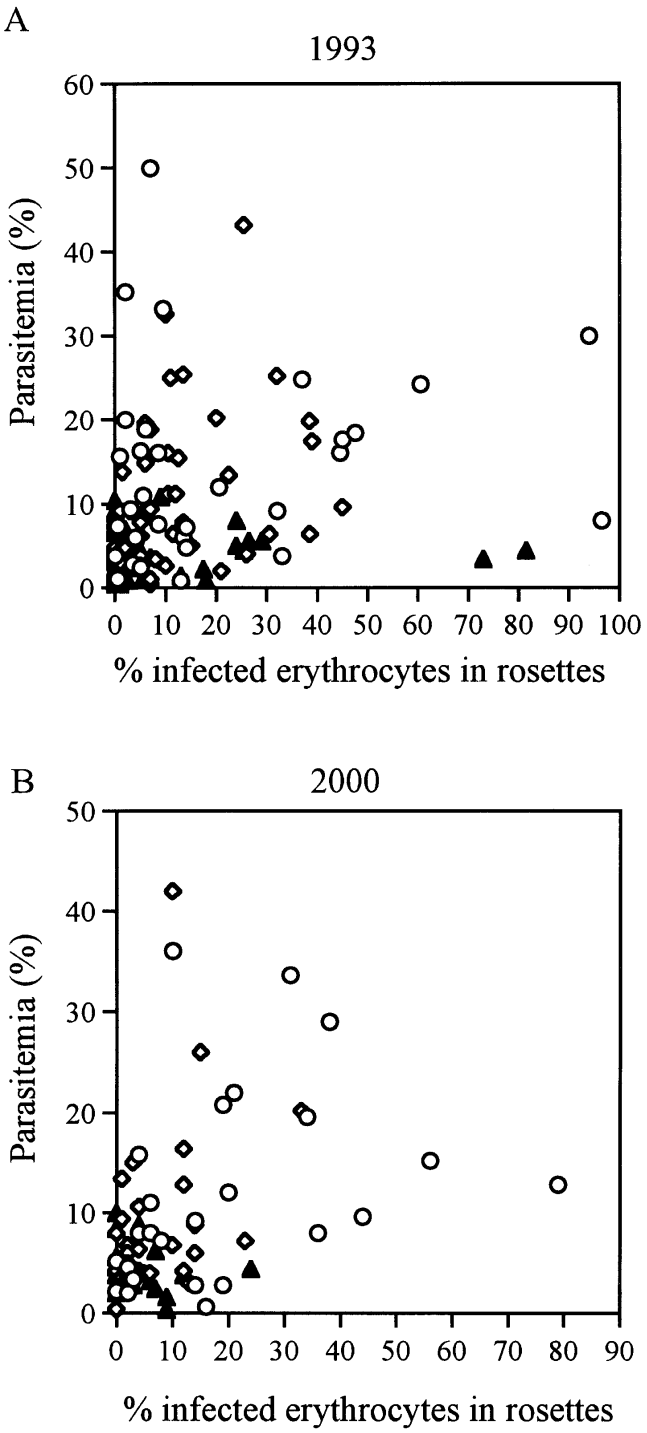

FIGURE 1. Scatter diagram of rosetting and parasitemia in *P. falciparum* clinical isolates from two studies in Kilifi, Kenya in **(A)** 1993 and **(B)** 2000. Isolates from severe cases are shown as white circles, moderate cases as white diamonds, and mild cases as black triangles.

The relationship between rosetting and parasitemia was then assessed in two separate studies in 1993 and 2000 using clinical isolates from children with severe, moderate, and mild malaria in Kilifi, Kenya. Blood samples were collected after informed consent from the children's parents or guardians, and ethical clearance was granted by the Kenya National Ethical Review Committee. Parasites were cultured using supplemented RPMI-1640 medium and 10% European AB serum, and rosetting was assessed by standard methods as described previously.[3] Patient data are provided in Table 1. The association between rosetting and severe malaria in these samples has been published elswhere.[3,16] Analysis of parasitemia and rosetting shows a moderate positive correlation between the two factors that is consistent with the hypothesis that rosetting enhances parasite growth and survival (Figure 1 and Table 1). Similar positive correlations between rosetting and parasitemia have been reported in studies from Thailand[6] and Papua New Guinea,[7] but no significant correlation was seen in the Gambia.[2] Interestingly, when the data were analyzed by clinical disease category, it was found in both 1993 and 2000 that the correlation between rosetting and parasitemia was only seen in children who were relatively sick (severe malaria and inpatient moderate malaria) and not in those with mild, uncomplicated cases (outpatient malaria) (see Table 1). The reason for this is unknown, although one could hypothesize that the rosettes seen in the outpatient cases are perhaps not functional in vivo either because the patients have rosette-disrupting antibodies[2] or red cell polymorphisms that reduce the stability of rosettes.[9,17] The precise role of rosette-disrupting antibodies in natural infections remains to be clarified because, although they were clearly demonstrated in a study in the Gambia,[2] we were unable to confirm the presence of such antibodies in Kenyan children.[3]

The correlation between rosetting and parasitemia shown in this study clearly does not prove a causal relationship between the two phenomena, because, for example, there could be a third, unidentified factor driving the relationship between these two variables. This work does, however, suggest that further investigations of the mechanisms by which rosetting may enhance parasite growth and survival are warranted. Parasite growth rates have been associated with malaria virulence in humans[18] and in animal models,[19] and it could be that rosetting is an important factor that influences the growth potential of different parasite isolates and in this way contributes to severe disease.

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