We thank Parola et al. (2006) for their comments on our article (Sasaki et al. 2006). Our response to their comments and criticisms are as follows.

First, Parola et al. (2006) comment that no information was given on negative controls. We reported some negative polymerase chain reaction (PCR) results in our article that served as de facto negative controls. Subsequently, whenever we used negative controls, no PCR products were ever obtained. Thus, we confirmed that our results were not due to contamination. Parola et al. (2006) also felt that nested PCR is hampered by a high risk of contamination. PCR results using single-use primers without positive controls were the same as nested PCR results. Our results were the same in the gltA gene assay. In addition, to prevent false positive reactions from surface contaminants, each louse was immersed for 5 min in a solution of 70% ethanol and 0.2% iodine and then washed for 5 min in sterile distilled water before DNA extraction. Every effort was made to eliminate any confusion due to contamination.

Second, Parola et al. (2006) comment that the validity of the morphological differentiation between head and body lice, based on leg length differences, is dubious. These two louse species were readily separated with the tibia length of their legs ($P < 0.002$) (data not shown). They are also identifiable by PCR using the NADH dehydrogenase subunit 4-encoding (ND4) gene (Raoult et al. 2006). We confirmed that our lice were indeed head lice by using a PCR-based identification of the ND4 gene.

One of us (S.K.S.P.) has shown that human head and body lice are genetically distinct using evidence from double infestations (Leo et al. 2005). Parola et al. (2006) also suggested that patients who are heavily infested with body lice also may have these ectoparasites on the head. However, in our study site in Nepal, there were no children heavily infested with body lice (Poudel and Barker 2004, Sasaki et al. 2006). Coauthor S.K.S.P. observed and took digital photographs to record the infestation status of all children with double infestations.

And third, B. quintana has never been detected in the laboratory of Parola et al. in thousands of head lice collected throughout the world. Our results obtained from Nepalese children are the first such finding, and it is related to poverty and unsanitary conditions. We confirmed our data again following the comments of Parola et al. (2006), and the same results were obtained. Based on our results, we continue to encourage readers to screen head lice as well as body lice for infection with B. quintana.

References Cited


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