A serosurvey for human ehrlichiosis caused by Ehrlichia chaffeensis and Anaplasma phagocytophilum was performed in different regions of Peru by using indirect immunofluorescence assays (IFAs). Regions included an urban community in a shantytown in Lima (Pampas) and three rural communities located on the northern coast of Peru (Cura Mori), in the southern Peruvian Andes (Cochapata), and in the Peruvian jungle region (Santo Tomas). An overall E. chaffeensis seroprevalence of 13% (21 of 160) was found by IFA. Seroprevalences in females and males was 15% (16 of 106) and 9% (5 of 53), respectively. Seroprevalences in Cura Mori, Cochapata, Pampas, and Santo Tomas were 25% (10 of 40), 23% (9 of 40), 3% (1 of 40), and 3% (1 of 40), respectively. Seroprevalences in Cura Mori and Cochapata were significantly higher than in Santo Tomas or Pampas (P < 0.01). No sera were reactive to A. phagocytophilum. These findings suggest that human infection with E. chaffeensis occurs in Peru. Further studies are needed to characterize Ehrlichia species in Peru, their vectors and their clinical significance.