Abstract: Abstract Background Plasmodium vivax accounts for about 40% of all malaria infection in Ethiopia. Chloroquine (CQ) is the first line treatment for confirmed P. vivax malaria in the country. The first report of CQ treatment failure in P. vivax was from Debre Zeit, which suggested the presence of chloroquine resistance. Methods An in vivo drug efficacy study was conducted in Debre Zeit from June to August 2006. Eighty-seven patients with microscopically confirmed P. vivax malaria, aged between 8 months and 52 years, were recruited and treated under supervision with CQ (25 mg/kg over three days). Clinical and parasitological parameters were assessed during the 28 day follow-up period. CQ and desethylchloroquine (DCQ) blood and serum concentrations were determined with high performance liquid chromatography (HPLC) in patients who showed recurrent parasitaemia. Results Of the 87 patients recruited in the study, one was lost to follow-up and three were excluded due to P. falciparum infection during follow-up. A total of 83 (95%) of the study participants completed the follow-up. On enrolment, 39.8% had documented fever and 60.2% had a history of fever. The geometric mean parasite density of the patients was 7045 parasites/μl. Among these, four patients had recurrent parasitaemia on Day 28. The blood CQ plus DCQ concentrations of these four patients were all above the minimal effective concentration (> 100 ng/ml). Conclusion Chloroquine-resistant P. vivax parasites are emerging in Debre Zeit, Ethiopia. A multi-centre national survey is needed to better understand the extent of P. vivax resistance to CQ in Ethiopia.