Studies were conducted to determine the role of sibling species of Anopheles funestus complex in malaria transmission in three agro-ecosystems in central Kenya. Mosquitoes were sampled indoors and outdoors, and rDNA PCR was successfully used to identify 340 specimens. Anopheles parensis (91.8%), A. funestus (6.8%), and Anopheles leesoni (1.5%) were the three sibling species identified. A. parensis was the dominant species at all study sites, while 22 of 23 A. funestus were collected in the non-irrigated study site. None of the 362 specimens tested was positive for Plasmodium falciparum circumsporozoite proteins by enzyme-linked immunosorbent assay. The most common blood-meal sources (mixed blood meals included) for A. parensis were goat (54.0%), human (47.6%), and bovine (39.7%), while the few A. funestus s.s. samples had fed mostly on humans. The human blood index (HBI) for A. parensis (mixed blood meals included) in the non-irrigated agro-ecosystem was 0.93 and significantly higher than 0.33 in planned rice agro-ecosystem. The few samples of A. funestus s.s. and A. funestus s.l. also showed a trend of higher HBI in the non-irrigated agro-ecosystem. We conclude that agricultural practices have significant influence on distribution and blood feeding behavior of A. funestus complex. Although none of the species was implicated with malaria transmission, these results may partly explain why non-irrigated agro-ecosystems are associated with higher risk of malaria transmission by this species compared to irrigated agro-ecosystems.