Waterborne diseases that originate from fecal pollution remain a significant public health issue. Current fecal indicator technologies recommended by the U.S. Environmental Protection Agency for water quality testing do not discriminate between different animal sources of fecal pollution. Real-time quantitative PCR (qPCR) applications represent a powerful tool for the identification and potential quantification of host-associated genetic markers. The concept of qPCR fecal source identification, as well as advantages, key challenges, and the application of this technology will be discussed.