The epidemiology and public health significance of Cryptosporidium species and genotypes were investigated in Beni-Suef Governorate, Egypt. A total of 610 animal fecal samples (480 from cattle and 130 from buffaloes) beside 290 stool samples from humans were collected in the period between January and December 2014. Based on the microscopic examination, the overall estimated prevalence of Cryptosporidium spp. in cattle, buffaloes, and humans was 10.2, 12.3, and 19 %, respectively. The highest detection rates were in calves less than 2 months of age (17.1 %) and diarrheic animals (13.0 %). Likewise in humans, the highest prevalence of Cryptosporidium was in infants (31.3 %) and diarrheic individuals (21.1 %). The gender distribution in humans denoted that Cryptosporidium was reported more frequently in males (21.7%) than females (14.5%). Based on the molecular characterization of Cryptosporidium, Cryptosporidium oocyst wall protein (COWP) and gp60 genes were successfully amplified in 36 out of 50 samples subjected to genotyping. Restriction fragment length polymorphism (RFLP) analysis of the COWP fragments revealed that Cryptosporidium parvum was the only species detected in cattle (12 isolates) and buffaloes (4 isolates), while in humans, the detected species were Cryptosporidium hominis (15 isolates) and C. parvum (5 isolates). Sequence analysis of the gp60 gene identified the subtype IIdA20G1 within C. parvum isolated from both animals and humans. The common occurrence of zoonotic subtypes of C. parvum in cattle and buffaloes highlights the potential role of these animals as significant reservoirs of infection to humans. Also, the presence of C. hominis and C. parvum in humans indicates that both anthroponotic and zoonotic pathways are expected.