Waterbuck (Kobus defassa), an ungulate species endemic to the Eastern African savannah, is suspected of being a wildlife reservoir for tick-transmitted parasites infective to livestock. Waterbuck is infested by large numbers of Rhipicephalus appendiculatus, the tick vector for Theileria parva, and previous data suggests that the species may be a source of T. parva transmission to cattle. In the present study, a total of 86 cattle and 26 waterbuck blood samples were obtained from Marula, a site in Kenya endemic for East Coast fever (ECF) where the primary wildlife reservoir of T. parva the Cape buffalo (Syncerus caffer) is also common. To investigate for the presence of cattle-infective Theileria parasites, DNA specimens extracted from the blood samples were subjected to two diagnostic assays; a nested PCR based on the p104 gene that is specific for T. parva, and a reverse line blot (RLB) incorporating 13 oligonucleotide probes including all of the Theileria spp. so far described from livestock and wildlife in Kenya. Neither assay provided evidence of T. parva or Theileria sp. (buffalo) infection in the waterbuck DNA samples. By contrast, majority of the cattle samples (67.4%) were positive for T. parva using a nested PCR assay. The RLB assay, including a generic probe for the genus Theileria, indicated that 25/26 (96%) of the waterbuck samples were positive for Theileria, while none of the 11 Theileria species-specific probes hybridized with the waterbuck-derived PCR products. Phylogenetic analysis of 18S ribosomal RNA (18S rRNA) and internal transcribed spacer (ITS) sequences within the RLB-positive waterbuck samples revealed the occurrence of three Theileria genotypes of unknown identity designated A, B and C. Group A clustered with Theileria equi, a pathogenic Theileria species and a causative agent of equine piroplasmosis in domestic equids. However, DNA from this group failed to hybridize with the T. equi oligonucleotide present on the RLB filter probe, suggesting the occurrence of novel taxa in these animals. This was confirmed by DNA sequencing that revealed heterogeneity between the waterbuck isolates and previously reported T. equi genotypes. Group B parasites clustered closely with Theileria luwenshuni, a highly pathogenic parasite of sheep and goats reported from China. Group C was closely related to Theileria ovis, an apparently benign parasite of sheep. Together, these findings provided no evidence that waterbuck plays a role in the transmission of T. parva. However, novel Theileria genotypes detected in this bovid species may be of veterinary importance.