Monitor lizards were sampled along the shores of Lake Victoria to detect natural infections of potentially human-infective trypanosomes. In an area with endemic rhodesian sleeping sickness, one of 19 lizards was infected (Busia, Kenya). Six of ten lizards also showed indirect evidence of infection with Trypanosoma brucei (antibody ELISA). In an area with no recent history of human disease (Rusinga Island), no parasites were found and no antibodies to T. brucei were detected. The isolate was identified as T. brucei through xenodiagnosis (completion of the life cycle in the salivary glands of tsetse), and through molecular techniques (positive reactions with a PCR primer and a microsatellite DNA probe characteristic of the subgenus Trypanozoon). Experimental infections of monitor lizards were also attempted with a variety of parasites and tsetse species. It was possible to infect monitor lizards with T. brucei but not with forest or savannah genotypes of Trypanosoma conglolense. Parasites reached low levels of parasitaemia for a short period without generating any pathology; they also remained infective to tsetse and laboratory rats. The implications of these findings are discussed in relation to the endemicity of sleeping sickness.