Chloramphenicol is a broad-spectrum antibiotic shown to have specific activity against a wide variety of organisms that are causative agents of several disease conditions in domestic animals. Chloramphenicol has been banned for use in food-producing animals for its serious adverse toxic effects in humans. Due to the harmful effects of chloramphenicol residues livestock products should be free of any traces of these residues. Several analytical methods are available for chloramphenicol analysis but sensitive methods are required in order to ensure that no traces of chloramphenicol residues are present in edible animal products. In order to prevent the illegal use of chloramphenicol, regulatory control of its residues in food of animal origin is essential. A competitive enzyme-linked immunosorbent assay for chloramphenicol has been locally developed and optimized for the detection of chloramphenicol in sheep serum. In the assay, chloramphenicol in the test samples and that in chloramphenicol-horseradish peroxidase conjugate compete for antibodies raised against the drug in camels and immobilized on a microtitre plate. Tetramethylbenzidine-hydrogen peroxide (TMB/H2O2) is used as chromogen-substrate system. The assay has a detection limit of 0.1 ng/mL of serum with a high specificity for chloramphenicol. Cross-reactivity with florfenicol, thiamphenicol, penicillin, tetracyclines and sulfamethazine was not observed. The assay was able to detect chloramphenicol concentrations in normal sheep serum for at least 1 week after intramuscular injection with the drug at a dose of 25 mg/kg body weight (b.w.). The assay can be used as a screening tool for chloramphenicol use in animals.