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Short communication

PROZONE REACTION IN AN ANTIBODY ELISA OF A BRUCELLOSIS POSITIVE DROMEDARY CAMEL SERUM

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The serological diagnosis of camel brucellosis uses routine serological tests described in details in the OIE (2018) which are mainly the Complement Fixation Test (CFT), the Serum Agglutination Test (SAT or TAT), Rose Bengal Test (RBT) and antibody ELISA. These tests were recently evaluated by Soellner *et al* (2018) for use in camelids, as each test should be validated for its fitness in the corresponding animal species (OIE, 2018). It is sometimes difficult to interpret the results as cross-reactivity with other bacterial species like *Yersinia enterocolitica,* serotype O9, may occur (Sunaga *et al*, 1983; Bisping and Amtsberg, 1988; Erdenebaatar *et al*, 2003) and with others like *Pasteurella, Campylobacter, Salmonella* and *Franciscella* (Markey *et al*, 2013). Zhulobovski and Pal'gov (1954) additionally described prozones in some sera of Bactrian camels in Russia and Nada (1984) in dromedaries in Egypt. The absence of a visual positive reaction in low serum dilutions has also been observed in 1.5% of all positive dromedary camel sera in the UAE when using Serum Agglutination Test (SAT, Wernery *et al*, 2014). The Coombs test is then necessary to verify the diagnosis of brucellosis in these cases. It is also proposed to add EDTA to the antigen which improves the test's specificity significantly (MacMillan and Cockrem, 1985). The prozone phenomenon occurs in agglutination or precipitation tests (Markey *et al*, 2013) and has until now not been described to occur with antibody ELISAs. The prozone phenomenon refers to a false negative serological response at low serum dilution due to excess antibody concentrations in the serum. The nature of this phenomenon is not entirely clear, but it is imperative that test sera be checked at several dilutions to avoid errors in reporting results. We report here a prozone phenomenon in one of 4 *Brucella*-positive dromedary camel sera with the competitive ELISA (c-ELISA) from Ingenasa, Spain (Table 1). All 4 sera were highly positive with the CFT, SAT, RBT and c-ELISA except serum number 4, which was negative in the ELISA. This serum was then diluted two-fold and it became positive in the ELISA at a dilution of 1:320. The prozone did not occur with the other 3 highly *Brucella*-positive sera and not at all in the agglutination test.

In conclusion, a dromedary camel serum which was highly positive in 3 serological tests for brucellosis, turned only positive in the antibody ELISA when it was diluted 1:320. To the knowledge of the authors, it is the first time that a prozone phenomenon was observed in a *Brucella*-positive camel serum when a c-ELISA was used.

Table 1. Serological brucellosis results of 4 dromedary camels using 4 different test methods.

ID	RBT	SAT	CFT	c-ELISA
1	+++*	1:1280	1:128 ++++*	Positive
2	+++	1:640	1:64 +++	Positive
3	++	1:320	1:4 ++++	Positive
4	++++	1:1280	1:256 ++++	Negative
				At a dilution of 1:320 positive

*= Score +++ : strong positive
++++: very strong positive

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