ABSTRACTS

ISCFR 2012

July 26-29, Whistler, Canada

7th International Symposium on Canine and Feline Reproduction

In a joint meeting with EVSSAR 2012

15th Congress of the European Veterinary Society for Small Animal Reproduction

Editors: Gary England, Michelle Kutzler, Pierre Comizzoli, Wojciech Nizanski, Tom Rijsselaere and Patrick Concannon

Reprinted in IVIS with the permission of the ISCFR Organizers
Comparison between a novel immunochromatography test and agglutination, agar-gel immunodiffusion and ELISAs for the serological diagnosis of canine brucellosis

Wanke, MM¹; Cairó, F¹; Rossano, M²; Laiño, M³; Baldi P⁴ and Martínez Vivot, M³

¹Theriogenology Area, ²INITRA, ³Infectious Diseases Area, Faculty of Veterinary Sciences, University of Buenos Aires, Chorroarín 280 (1427) CABA Argentina, and ⁴IDEHU, School of Pharmacy and Biochemistry, University of Buenos Aires, Junín 956, Buenos Aires, Argentina
wanke@fvet.uba.ar.

OBJECTIVES: As in other species, the diagnosis of brucellosis in the dog continues to be problematic. The most widely used screening test is the rapid slide agglutination test in the presence of 2-mercaptoethanol (2ME-RSAT) using whole cells of the M(-) strain of Brucella canis as antigen. The diagnosis is partially confirmed by agar-gel immunodiffusion test (AGID) and definitively confirmed by bacteriological isolation. Some chronic cases that may not be detected by these tests may be detected by ELISA tests that use a hot-saline extract (HS) or cytosolic proteins (CP) of Brucella as antigens. The use of 2ME-RSAT in the routine clinical practice is complicated by the need of a microscope to read the reaction and an experienced operator to interpret the results. An immunochromatographic diagnostic test for canine brucellosis (FASTest® Brucella c., Megacor, Hörbranz, Austria) has been recently released, which is very simple to perform and could be potentially used in the routine clinical practice. In the present study we compared the diagnostic performance of the FASTest with those of 2ME-RSAT, AGID and ELISAs.

MATERIALS AND METHODS: Sera: 50 sera were used which included 17 sera from healthy dogs, 27 sera from dogs with acute or subacute brucellosis confirmed by B. canis isolation, and 6 sera from dogs with bacteriologically confirmed chronic brucellosis.

Tests: all the samples were tested by RSAT, AGID, HS-ELISA, CP-ELISA and FASTest®.

RSAT and 2-ME-RSAT: all the samples were analyzed by RSAT (1), using a suspension of B. canis M- (Brucellosis service, ANLIS-Malbrán, Buenos Aires). The strength of agglutination was scored from + to ++++. Samples positive by RSAT were assayed by 2ME-RSAT.

AGID: a hot-saline extract of B. ovis (SENASA, Argentina) was used as antigen and the test was performed according to the Manual of Standards for Diagnostic Tests and Vaccines (2). The positive control serum was also provided by SENASA. ELISAs: Two ELISA tests were used, one with HS from B. canis (M-) as antigen, obtained as described elsewhere (3) and the other using the CP fraction of B. abortus depleted of LPS as described previously (4). Serum samples diluted 1:200 were dispensed on antigen-coated wells, and after 1 h of incubation and several washes an HRP-conjugated anti-dog IgG serum was added. The reaction was developed 1 h later by the addition of OPD/H2O2 substrate. Reactions were read at 490 nm. To determine the cut-off value of each assay, 50 sera from healthy dogs were tested under the same conditions that samples, and the cut-off value was calculated as mean OD plus 3 SD.

Immunochromatography: a commercial test (FASTest® Brucella c., Megacor, Hörbranz, Austria) was used. A drop of the serum sample or positive control serum is dispensed in the cassette using the pipette provided in the kit, followed by 4 drops of diluent. The reaction is read 20 minutes later. If a pink-purple line appears in the CONTROL zone (C) and no pink-purple line is visible in the TEST zone (T), the result is negative, and if a clearly visible pink-purple line also appears in the TEST zone (T), no matter which band appears first indicates a positive result.

RESULTS: Sera from 17 healthy dogs used as negative controls yielded negative results by FASTest, indicating a 100% specificity in this sample. Among 27 sera from dogs with acute or subacute brucellosis confirmed by B. canis isolation (all of which were positive by RSAT and ELISAs) the FASTest was positive in 24 cases and AGID in 23. In the 3 dogs negative by FASTest the test was repeated with cassettes from other lot, and a positive result was obtained in 2 cases. Sera from 6 dogs with bacteriologically confirmed chronic brucellosis which were positive by ELISAs but negative by 2ME-RSAT were also tested; 1 resulted positive by FASTest and 4 by AGID.

DISCUSSION: These preliminary results indicate a good specificity of the FASTest (100% in this sample). In acute and subacute cases the sensitivity of the test was 89% in the initial run and 96% if the re-assayed samples are considered. This later value, however, would not be statistically acceptable since the test was repeated due to the uncertain performance of a specific lot and on the basis of previously known results. In cases with chronic brucellosis the sensitivity of the FASTest was lower than that of ELISAs.
CONCLUSION: While this preliminary evaluation of the FASTest yielded promising results, it would be necessary to test a higher number of samples at least in duplicate to draw sound conclusions on the reliability of this test as a screening tool for canine brucellosis.

(1) Carmichael LE, Joubert JC. A rapid slide agglutination test for the serodiagnosis of *Brucella canis* infection that employs a variant (M-) organism as antigen. The Cornell Veterinarian. 1987; 77:3-12.

