Serum samples can be substituted by plasma samples for the diagnosis of paratuberculosis

Article *in* Preventive Veterinary Medicine · July 2013

DOI: 10.1016/j.prevetmed.2013.06.011 · Source: PubMed

Preventive Veterinary Medicine 112 (2013) 147–149

Contents lists available at SciVerse ScienceDirect

Preventive Veterinary Medicine

journal homepage: www.elsevier.com/locate/prevetmed

Short communication

Serum samples can be substituted by plasma samples for the diagnosis of paratuberculosis

Amador Goodridge[a,*], Ricardo Correa[a], Paul Castro[b], Cecilia Escobar[c], Jacobus H. de Waard[d]

[a] Instituto de Investigaciones Científicas y Servicios de Alta Tecnología INDICASAT-AIP, City of Knowledge, Panama
[b] Programa de Zootecnia, Universidad de Cundinamarca, Fusagasuga, Colombia
[c] Instituto de Investigaciones Agropecuarias de Panamá – IDIAP, City of Knowledge, Panama
[d] Laboratorio de Tuberculosis, Instituto de Biomedicina, Universidad Central de Venezuela, Caracas, Venezuela

ARTICLE INFO

Article history:
Received 24 March 2013
Received in revised form 3 June 2013
Accepted 23 June 2013

Keywords:
Bovine tuberculosis
Paratuberculosis
Diagnosis
ELISA
PARACHECK®
Biomarker

ABSTRACT

Employing plasma samples rather than serum samples for serological paratuberculosis diagnosis is practical, especially when bovine TB is assessed in the same cattle herd with the gamma interferon bovine avian (IFN-γ BA) test. We demonstrate that antibody titers in serum and plasma samples, utilizing the PARACHECK® ELISA kit, are highly comparable (Cohen's kappa test, $k = 0.955$). We conclude that serum can be replaced with plasma in this commercially available antibody detection assay resulting in working hour savings for sampling and blood sample work-up and cost reductions for materials and sample storage.

1. Introduction

Bovine tuberculosis (bTB) and paratuberculosis (PTB) (Johne's disease), caused by *Mycobacterium bovis* and *M. avium* subsp. *paratuberculosis* (MAP), respectively, are major infectious diseases among cattle (Ott et al., 1999). Methods presently used for bTB diagnosis include the interferon-γ assay, which is commercially available as an ELISA kit under the name BOVIGAM® (Wadhwa et al., 2012). The prevalence of PTB in a herd can be assessed through serology, and several commercial ELISA kits are available (Shin et al., 2008).

The interferon-γ assay utilizes heparinized plasma samples, whereas the ELISA kits available for PTB diagnosis recommend using serum samples. As both diagnostic methods are often used in parallel on the same animal or cattle herd, two blood samples have to be drawn into separate tubes, resulting in extra working hours both during sampling and sample work-up and extra costs in materials and sample storage. As a consequence, the cost of monitoring programs for cattle farms increases when using these two sample types.

Serum differs from plasma in that the former does not contain fibrinogen and clotting factors. Although serum and heparinized plasma specimens are considered equivalent for many assays, differences between these two sample types have been reported for several clinical biochemical analyses, such as albumin, alkaline phosphatase, calcium, carbon dioxide, chloride, creatine kinase, glucose, lactate dehydrogenase (LD), inorganic phosphorus, potassium, and total protein (Dossus et al., 2009); these differences are large enough to affect clinical interpretation in certain instances. In addition, it has been demonstrated that serum and plasma samples show poor correlations for the cytokines IL-6, TNF-alpha and IL-1beta ($r < 0.40$), but very high correlations were obtained for c-reactive protein (CRP) ($r = 0.98$) and fairly good correlations were obtained for IL-1Ra ($r = 0.60$) (Dossus et al., 2009).

* Corresponding author. Tel.: +507 517 0738; fax: +507 517 0701.
E-mail address: amadorgj@yahoo.com (A. Goodridge).

0167-5877/$ – see front matter © 2013 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.prevetmed.2013.06.011

Few studies have correlated antibody titers to microbial antigens in simultaneously obtained serum and plasma samples. One study, using a commercial test, showed a strong and statistically significant correlation between the serum and plasma IgG antibody responses to a herpes simplex virus glycoprotein in a sexually active woman (Cherpes et al., 2003). However, in a study with serum and plasma samples from patients for the validation of two commercially available ELISAs to detect carbonic anhydrase (CA), one of the ELISAs revealed significant differences in measured CA concentrations between EDTA plasma and serum. This appeared to be due to a metalion-dependent epitope on CA recognized by the detection antibody in this particular assay. The other commercially available ELISA was successfully validated and showed no difference in CA detection between EDTA plasma and serum (Wind et al., 2011). This study highlights the need for stringent validation of commercially available ELISA assays, including examination of various sample types, before use in research studies.

No validation studies have been published comparing serum and plasma samples for PTB diagnosis, and the PARACHECK® kit instruction manual recommends using only serum samples. Only one study was found after PUBMED search, in which this kit was utilized to examine the humoral immunity in sheep after vaccination; however without validating the use of plasma samples (Reddacliff et al., 2006). The present study was undertaken to determine whether plasma samples can be used instead of serum samples for bovine PTB diagnosis.

2. Materials and methods

In February 2013, a total of 209 cows from a dairy farm in the Coclé region of Panama were tested for PTB using the PARACHECK® ELISA test (Prionics AG, Switzerland) according to the manufacturer's protocol. The study herd included 22 (49%) Gyr breed cows, 22 (49%) Jersey cows and one (2%) Mestizo cow. Blood samples were obtained using Vacutainer® tubes containing lithium heparin for plasma and Vacutainer® tubes with no additive for serum samples. All tubes were centrifuged for 5 min at 4000 rpm to separate the cells, and the serum or plasma was stored for two days at 4 °C before performing the ELISAs. A subset of 45 plasma and serum samples from animals that were positive ($n = 19$) and negative for PTB ($n = 26$), as determined by the PARACHECK® ELISA using serum samples, were used to evaluate the assay correlations. Serum and plasma samples from the same animal were loaded in pairs in one PARACHECK® ELISA plate, together with two negative and two positive controls provided by the kit manufacturer. IBM SPSS statistics software version 20 (IBM, New York, USA) was used for statistical analysis. The serum and plasma antibody titers determined by optical density (O.D.) readings were compared using the Pearson's correlation test. The agreement between the two sample types was determined by Cohen's kappa analysis. A linear mixed effects model was used to examine the relationship between results obtained from the serum and plasma samples while adjusting for breed and age. Inter-assay variability was assessed using the intra-class correlation coefficient (ICC) calculated from the random effects model. This approach is appropriate for repeated measures from the same animal when measurement independence cannot be assumed. Log-transformed O.D. readings were entered into the model as the dependent variable.

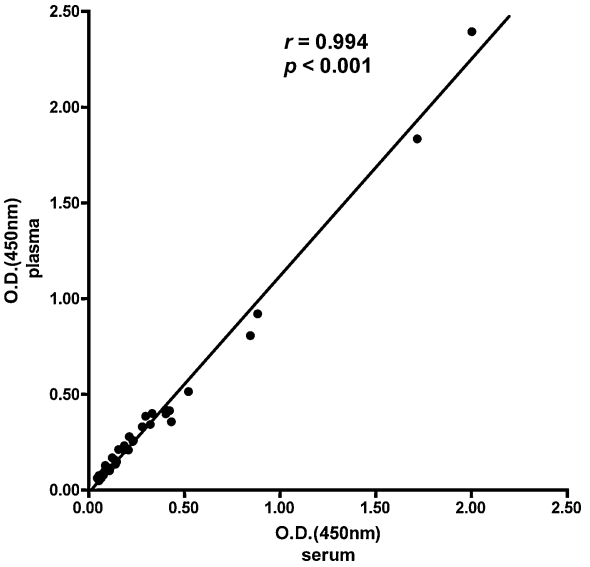

Fig. 1. Correlation graph for the assay of serum and plasma antibody titers for the diagnosis of bovine paratuberculosis in the PARACHECK ELISA. The Pearson's correlation was used to test for statistical significance.

3. Results

The 45 selected cows were all female, with an average age of 48 months (range 18–139 months). Two of the cows were pregnant and none had PTB symptoms. The average age of the Jersey breed cows was 40 months old (95% CI = 31.7–48.1), while the average age of the Gyr cows was 78 months old (95% CI = 63.4–92.3). Forty-three (95.6%) animals were born outside of Panama. Of the selected cows, 42% (19/45) had a positive PTB result for the PARACHECK® ELISA using a serum sample. The O.D. readings were non-significantly elevated in serum samples for both cow breeds. Jersey breed cows showed an average O.D. reading at 0.210 in serum vs. 0.184 in plasma, while Gyr breed showed O.D. readings of 0.417 vs. 0.386, respectively. Both serum and plasma sample O.D. reading results for the PARACHECK® test showed a strong Pearson's correlation coefficient ($r = 0.994$, 95% CI = 0.989–0.997) (Fig. 1). Although, our sample set contained two samples with extremely high O.D. values (O.D. > 1.5) with sera; the correlation between the serum and plasma values remained strong at 0.989 when these two values were excluded from the analysis. There was almost perfect agreement between the serum- and plasma-based diagnosis of PTB (Cohen's $\kappa = 0.955$). We also observed positive correlation between age and O.D. readings ($r = 0.360$, $p < 0.001$). It is worth noting that the mean O.D. value for the serum sample group was slightly elevated, but the difference between the serum and plasma group values was not statistically significant (0.305 ± 0.440 vs. 0.277 ± 0.394, respectively; $p = 0.750$,

Student's *t*-test). However, the linear mixed model analysis revealed a significant relationship between breed and O.D. readings ($p < 0.001$) with a magnitude (β) of 0.457 (95% CI = 0.245–0.669). The estimated ICC coefficient between serum and plasma samples was 0.91 (95% CI = 0.87–0.93).

4. Conclusion

Our findings support the interchangeability between serum and plasma samples for measuring antibodies against MAP using the PARACHECK® ELISA kit. Substitution of serum for plasma samples will facilitate PTB control by reducing material and labor costs, and bovine tuberculosis can be assessed in a cattle herd at the same time. For example, one sample can be collected and divided for interferon-γ and MAP antibody detection. In addition, plasma samples have been shown to be useful for identifying new PTB infection and disease biomarkers (You et al., 2012) and can be stored for this purpose. Our findings are limited to the ELISA kit used in this study. Evaluation of the use of plasma with other ELISA kits is recommended since variations in diagnostic performance may depend on the kit manufacturer. Kits have shown different sensitivities and specificities when applied to samples from the same cattle herd (Collins et al., 2005; McKenna et al., 2005a,b), as different antigen preparations are used in the different ELISAs. For example, in samples obtained from human patients, a significant and strong correlation between serum and plasma antibody titers to different mycobacterial antigens was demonstrated (Siev et al., 2011). However, the correlation coefficient reported by Siev et al. was dependent on the mycobacterial protein used in the ELISA. In this study, the Spearman's correlation values between serum and plasma responses varied between 0.88 and 0.99 depending on the antigen. Although our study suggests that it is possible to use plasma samples to diagnose PTB, and thereby reduce labor and material costs, future studies are required to determine the estimated correlation within known PTB-negative and PTB-positive cohorts to further validate this change in protocol.

Funding

This research was funded partially by the National Secretariat of Science and Technology of Panama (SENACYT) Tuberculosis Biomarker Grant IDR-10-067 and FUNDAIM (Fundación para la investigación en Micobacterias), Caracas, Venezuela.

Conflicting interests

Authors declare no conflict of interest.

Acknowledgements

The authors thank Inversiones para el Desarrollo de Coclé (INDECO) for providing access to the dairy production cattle herd for this research and for providing the commercial diagnostic kits. We also thank Dr. Santiago Alvarez, the lead veterinarian at the dairy farm, for his collaboration. We thank Juan Carlos Montano and Selma Franco for their assistance in collecting the blood samples. We also thank Howard Takiff and Colleen Goodridge for helpful edits and Gabrielle Britton for her assistance in the statistical analysis.

References

Cherpes, T.L., Meyn, L.A., Hillier, S.L., 2003. Plasma versus serum for detection of herpes simplex virus type 2-specific immunoglobulin G antibodies with a glycoprotein G2-based enzyme immunoassay. J. Clin. Microbiol. 41, 2758–2759.

Collins, M.T., Wells, S.J., Petrini, K.R., Collins, J.E., Schultz, R.D., Whitlock, R.H., 2005. Evaluation of five antibody detection tests for diagnosis of bovine paratuberculosis. Clin. Diagn. Lab. Immunol. 12, 685–692.

Dossus, L., Becker, S., Achaintre, D., Kaaks, R., Rinaldi, S., 2009. Validity of multiplex-based assays for cytokine measurements in serum and plasma from non-diseased subjects: comparison with ELISA. J. Immunol. Methods 350, 125–132.

McKenna, S.L., Keefe, G.P., Barkema, H.W., Sockett, D.C., 2005a. Evaluation of three ELISAs for *Mycobacterium avium* subsp. *paratuberculosis* using tissue and fecal culture as comparison standards. Vet. Microbiol. 110, 105–111.

McKenna, S.L., Sockett, D.C., Keefe, G.P., McClure, J., VanLeeuwen, J.A., Barkema, H.W., 2005b. Comparison of two enzyme-linked immunosorbent assays for diagnosis of *Mycobacterium avium* subsp. *paratuberculosis*. J. Vet. Diagn. Invest. 17, 463–466.

Ott, S.L., Wells, S.J., Wagner, B.A., 1999. Herd-level economic losses associated with Johne's disease on US dairy operations. Prev. Vet. Med. 40, 179–192.

Reddacliff, L., Eppleston, J., Windsor, P., Whittington, R., Jones, S., 2006. Efficacy of a killed vaccine for the control of paratuberculosis in Australian sheep flocks. Vet. Microbiol. 115, 77–90.

Shin, S.J., Cho, D., Collins, M.T., 2008. Diagnosis of bovine paratuberculosis by a novel enzyme-linked immunosorbent assay based on early secreted antigens of *Mycobacterium avium* subsp. *paratuberculosis*. Clin. Vaccine Immunol. 15, 1277–1281.

Siev, M., Yu, X., Prados-Rosales, R., Martiniuk, F.T., Casadevall, A., Achkar, J.M., 2011. Correlation between serum and plasma antibody titers to mycobacterial antigens. Clin. Vaccine Immunol. 18, 173–175.

Wadhwa, A., Hickling, G.J., Eda, S., 2012. Opportunities for improved serodiagnosis of human tuberculosis, bovine tuberculosis, and paratuberculosis. Vet. Med. Int. 2012, 674238.

Wind, T.C., Messenger, M.P., Thompson, D., Selby, P.J., Banks, R.E., 2011. Measuring carbonic anhydrase IX as a hypoxia biomarker: differences in concentrations in serum and plasma using a commercial enzyme-linked immunosorbent assay due to influences of metal ions. Ann. Clin. Biochem. 48, 112–120.

You, Q., Verschoor, C.P., Pant, S.D., Macri, J., Kirby, G.M., Karrow, N.A., 2012. Proteomic analysis of plasma from Holstein cows testing positive for *Mycobacterium avium* subsp. *paratuberculosis* (MAP). Vet. Immunol. Immunopathol. 148, 243–251.