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Validation of a Paraoxon-based method for measurement of Paraoxonase (PON-1) Activity and establishment of RI in horses

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Abstract

Paraoxonase-1 (PON-1) is an anti-oxidant compound considered as negative acute phase protein in animals (Rossi *et al.*, 2013) and people (Novak *et al.*, 2010). The paraoxon-based method for measurement of PON-1 in equine serum has not yet been validated.

The aim of this study is to validate a paraoxon-based method to measure PON-1 and to establish reference intervals (RIs) in healthy horses and foals.

120 horses (40 geldings, 40 stallions, 40 mares; median age: 11 years; 57 Warmbloods, 46 Trotters) and 55 foals (27 females, 28 males; median age: 47 days; 22 Warmbloods, 31 Trotters) considered healthy after physical examination and biochemistry were examined. Horses were grouped by breed: Thoroughbreds, Trotters, Warmbloods, Draft horses and Ponies. Serum PON-1 was measured with an automated spectrophotometer and an enzymatic method validated in other species (Giordano *et al.*, 2013). After the analytical validation (precision, accuracy, interference studies), RIs were determined using the Reference Value Advisor software, according to ASCVP guidelines (Friedrichs *et al.*, 2012). The possible gender-, age- and breed-related differences were statistically investigated.

The paraoxon-based method was precise (CVs <4.0%) and accurate (P<0.001 in linearity under dilution and spike-recovery testing) but is affected by interference from mild bilirubinemia, severe lipemia or hemoglobinemia. The RIs recorded in the whole population was 38.1-80.8 U/mL. According to the Harris and Boyd test, separate RIs are recommended only for adult females and for Warmblood and Trotter adults (Figure 1).

This study demonstrated that analytical performances of the paraoxon-based method for measurement of PON-1 in horses are acceptable. PON-1 is lower in horses than in other species.

If future studies will demonstrate that oxidative stress induces a significant decrease of PON-1, this results will be useful to correctly classify healthy and sick horses; PON-1 could be used, as in human medicine, as a marker of oxidative stress.

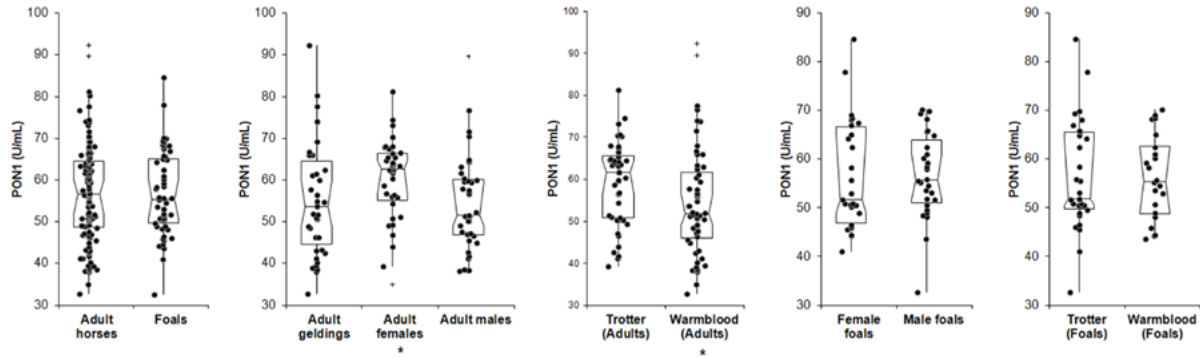


Fig. 1: Comparison of results obtained in adult horses vs foals, stallions vs mares vs geldings, Trotters vs Warmblood adult horses, male vs female foals, and Trotter vs Warmblood foals. The boxes indicate the I–II interquartile range (IQR), the horizontal line indicates the median values, whiskers extend to further observation within quartile I minus $1.5 \times$ IQR or to further observation within quartile III plus $1.5 \times$ IQR. ‘+’ indicates near outliers (i.e., values exceeding quartiles I or III minus or plus $1.5 \times$ IQR). The asterisk indicates a significant difference (mares vs stallions and geldings; adult Trotters vs Warmbloods).

References

- Friedrichs, K.R., Harr, K.E., Freeman, K.P., Szladovitz, B., Walton, R.M., Barnhart, K.F., Blanco-Chavez, J. 2012. ASVCP reference interval guidelines: determination of de novo reference intervals in veterinary species and other related topics. *Vet Clin Pathol.* 41, 441-453.
- Giordano, A., Veronesi, M.C., Rossi, G., Pezzia, F., Probo, M., Giori, L., Paltrinieri, S., 2013. Serum paraoxonase-1 activity in neonatal calves: age related variations and comparison between healthy and sick animals. *Vet J.* 197, 499-501.
- Novak, F., Vavrova, L., Kodydkova, J., Novak, F.S., Hynkova, M., Zak, A., Novakova, O. 2010. Decreased paraoxonase activity in critically ill patients with sepsis. *Clin Exp Med.* 10, 21-25.
- Rossi G, Giordano A, Pezzia F, Kjelgaard-Hansen M, Paltrinieri S. 2013. Serum paraoxonase 1 activity in dogs: preanalytical and analytical factors and correlation with C-reactive protein and alpha-2-globulin. *Vet Clin Pathol.* 42, 329-341.