Research in Veterinary Science 158 (2023) 44–49

Contents lists available at ScienceDirect

Research in Veterinary Science

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

Measurement of haptoglobin in saliva of cows: Validation of an assay and a pilot study of its potential application

M. Botía [a], M. López-Arjona [b], D. Escribano [a,c], M.D. Contreras-Aguilar [a], P.J. Vallejo-Mateo [d], J.J. Cerón [a], S. Martínez-Subiela [a,*]

[a] Interdisciplinary Laboratory of Clinical Analysis, Interlab-UMU, Regional Campus of International Excellence Campus Mare Nostrum, University of Murcia, 30100 Murcia, Spain
[b] Department of Animal and Food Science, Universitat Autònoma de Barcelona, 08193 Bellaterra, Spain
[c] Department of Animal Production, Veterinary School, Regional Campus of International Excellence Campus Mare Nostrum, University of Murcia, 30100 Murcia, Spain
[d] Department of Animal Medicine and Surgery, Veterinary School, Regional Campus of International Excellence Campus Mare Nostrum, University of Murcia, 30100 Murcia, Spain

ARTICLE INFO

Keywords:
Dairy cattle
Haptoglobin
Lameness
Peripartum
Saliva

ABSTRACT

In recent years, the use of saliva as a matrix for the measurement of biomarkers of health and welfare is gaining importance due to its non-invasive collection. Haptoglobin (Hp) is an acute-phase protein involved in the inflammatory response and changes in its concentration can provide information about the health status of the animals. This study aimed to develop and validate an assay based on luminescent amplification (AlphaLISA technology) for the measurement of Hp in bovine saliva and to study the possible changes in different inflammatory situations such as peripartum period and lameness.

The assay proved to be accurate, reliable, and sensitive for the measurement of Hp in cow saliva (coefficient of variation (CV) 7.57%; coefficient of determination (R^2) 0.992; recovery test 105.15%; lower limit of quantification (LLQ) 7.9 ng/ml). Significant differences were observed between Hp levels in saliva of cows before (13 days before) and after (7 and 20 days after) calving and at the moment of calving ($p < 0.0001$), and between lame and healthy cows ($p < 0.008$). In conclusion, this assay can detect Hp in a precise, sensitive, and accurate way in saliva of cows. Future studies with a larger population and different disease conditions should be conducted to determine the potential of Hp as an inflammatory biomarker in cow saliva.

1. Introduction

Haptoglobin (Hp) is an acute phase protein (APP) synthesized mainly in the liver and lungs (Naryzhny and Legina, 2021). Some stimuli such as bacterial endotoxins or inflammatory processes lead to the release of cytokines such as IL-6, which is the main inducer of Hp synthesis (di Masi et al., 2020; Quaye, 2008). In this way, Hp is considered a marker of infection and inflammation, increasing in these conditions. In addition, it has a high specificity for binding to free hemoglobin and forming complexes that are rapidly eliminated, preventing the oxidative action of hemoglobin (Andersen et al., 2017; Levy et al., 2010). Thus, in hemolysis, Hp levels can drop, being considered also as an indicator of this process (Shih et al., 2014). Other functions of Hp have been described such as activation of the anti-inflammatory response by binding to macrophages, a bacteriostatic effect by making iron unavailable to bacteria or an angiogenesis and chaperone activity (Ceciliani et al., 2012).

In humans, serum Hp ranges from 0.38 to 2.27 g/l in physiologic conditions, with up to three different phenotypes in circulation (Simon et al., 2020). It is considerate a moderate APP, and it is used for detection of inflammation, hemolytic disease and certain types of cancers (Lu et al., 2016; Marchand et al., 1980; Shu et al., 2010). In the veterinary field Hp is a major APP in cows, while in other species such as swine or dogs it is considered a moderate APP (López-Arjona et al., 2021). Healthy cows have very low to undetectable values of Hp in serum, so any elevation of its concentration can indicate an inflammatory process (Smith et al., 2010). The samples used more frequently for its measurement are serum and milk (Chan et al., 2004; Nirala et al., 2020; Simões et al., 2018; Smith et al., 2010). In this species, serum Hp is used for the early detection of inflammatory conditions such as mastitis

* Corresponding author.
E-mail address: silviams@um.es (S. Martínez-Subiela).

https://doi.org/10.1016/j.rvsc.2023.03.005
Received 1 February 2023; Accepted 6 March 2023
Available online 9 March 2023

(Simões et al., 2018; Wollowski et al., 2021).

In recent years, saliva has been gaining importance in cows as a matrix for the measurement of different biomarkers such as salivary alpha-amylase and butyrylcholinesterase (stress biomarkers), adenosine deaminase A (immunity biomarker) or oxytocin (positive emotions biomarker) (Contreras-Aguilar et al., 2019; López-Arjona et al., 2021). Since saliva samples can be obtained by a non-invasive and non-painful technique without specialized staff contrary to what happens with venepuncture for blood collection (Burnett et al., 2014; Tvarijonaviciute et al., 2020). Other APPs such as serum amyloid A have been measured in saliva of cows (Caplen and Held, 2021). However, to the author's knowledge, Hp has not been measured in bovine saliva.

Thus, this study aimed (1) to investigate if Hp could be analyzed in cow saliva and (2) to perform a pilot study to evaluate whether its concentration in saliva can change in situations of inflammation. For those purposes, a specific method for measuring Hp in cow saliva was developed and validated, and its possible changes in cows around the partum and lameness were evaluated.

2. Materials and methods

2.1. Development and optimization of the assay for Hp measurement

The assay was performed using AlphaLISA technology (PerkinElmer, Inc., MA, USA), based on amplified luminescence by the proximity of donor and acceptor beads. The explicative scheme of the assay for the detection of Hp in saliva is shown in Fig. 1. For the measurement of Hp in bovine saliva, a direct sandwich assay was developed in 96-well plates (PerkinElmer, Inc., MA, USA) with a total volume of 50 µl per well. The assay was performed with a commercial monoclonal antibody against Hp (HAPT-11-3F7, Life Diagnostics, Inc. PA, USA) conjugated to 1 mg of acceptor beads (AlphaScreen® Unconjugated Acceptor Beads, PerkinElmer, MA, USA); and a commercial polyclonal antibody against Hp (18,120, Life Diagnostics, Inc. PA, USA) biotinylated with a 60-fold molar excess (EZ-Link™, Micro Sulfo-NHS-Biotin, No-Weight™ Format, Thermo Scientific, USA). To optimize the assay, different concentrations of biotinylated antibody (0, 0′3, 0′6, 1, 3, and 6 nM) were tested; for the other components, the manufacturer's recommendations were used. A cow serum sample of known concentration (1.28 g/l) measured with a previously validated method (Eckersall et al., 1999) was used as standard, and the curve was generated with 8 standards at concentrations of 533, 266, 133, 67, 33, 17, 8 and 0 ng/ml. Finally, three types of buffers (Alpha buffer, universal buffer, and hi-block buffer from Perkin Elmer, Inc., MA, USA) were also tested for the measurement of the standard curve and different samples. The results are expressed in ng/ml. The conditions that finally proved to be optimal for the Hp measurement assay in cow saliva are shown in Fig. 2.

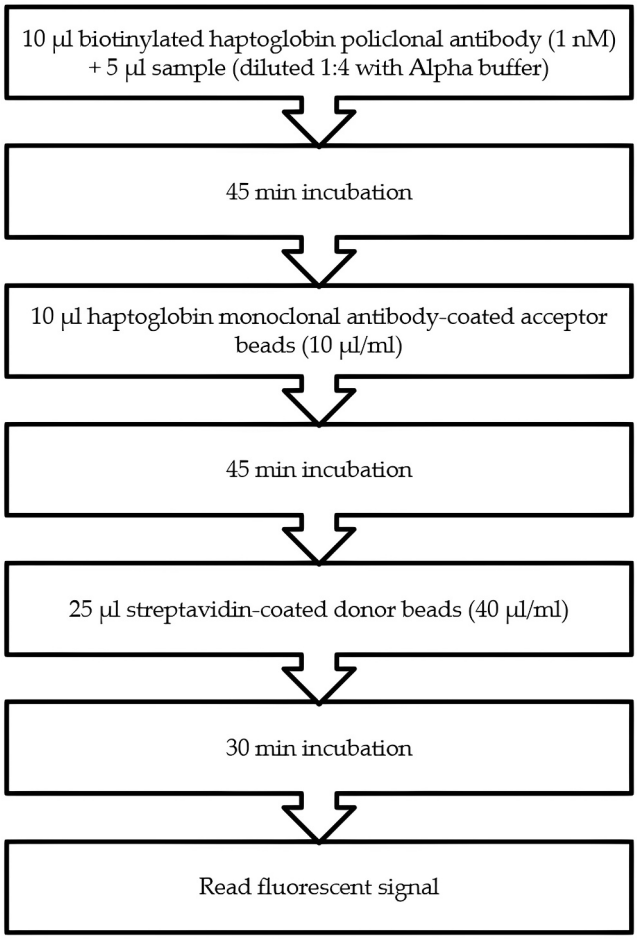

Fig. 2. AlphaLISA protocol for saliva haptoglobin (Hp) measurement in cows.

2.2. Analytical validation of the assay

2.2.1. Precision

The precision of the method was evaluated by the intra- and inter-assay coefficients of variation (CV). For intra-assay precision, 3 pools of samples of different concentrations (high, medium, and low concentration) were prepared and 5 replicates of each were measured, as described by López-Martínez et al., 2022. For inter-assay precision, 5 aliquots from each pool were stored at −80 °C, and each was measured in duplicate on five different days, each time using freshly prepared

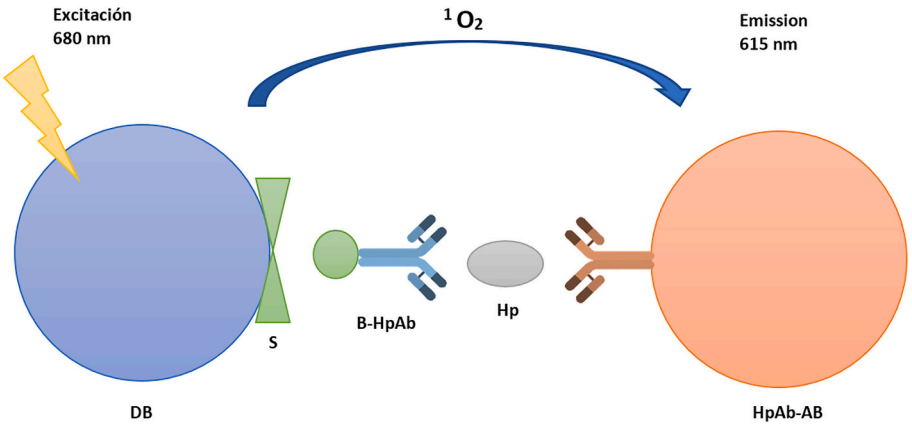

Fig. 1. Explicative scheme of the AlphaLISA reaction for haptoglobin detection. DB, donor bead; S, streptavidin; B-HpAb, biotinylated anti-haptoglobin polyclonal antibody; Hp, haptoglobin; Ab-HpAb, anti-haptoglobin monoclonal antibody conjugated to acceptor bead.

standard curves.

2.2.2. Accuracy

A linearity under dilution test was performed on two samples with high Hp concentration, as described by Escribano et al., 2012. The samples were serially diluted from 1:4 to 1:64 with the assay buffer. A recovery test was also made by adding different dilutions (from 1:4 to 1:64) of a cow saliva sample of known concentration (890.8 ng/ml) to two samples with high (892 ng/ml) and low (128 ng/ml) concentration with constant dilution (1:4).

2.2.3. Sensitivity

The limit of detection (LD) and lower limit of quantification (LLQ) were obtained to evaluate the sensitivity of the method. The LD was calculated as the mean of 15 replicate measurements of the assay buffer plus three standard deviations. For the LLQ, a serial dilution (from 1:4 to 1:256) of a high Hp concentration (669.2 ng/ml) cow saliva sample was performed, analyzing 5 replicates of each dilution. The CV was calculated for each dilution, establishing as LLQ the lowest dilution that can be repeatedly measured with a CV <20% (López-Arjona et al., 2020).

2.2.4. Changes in Hp during peripartum

A total of 48 saliva samples from a previous report were used for the evaluation of changes in salivary Hp during peripartum (Contreras-Aguilar et al., 2021). Saliva samples were taken from 12 Holstein-Friesian multiparous cows in late gestation from a commercial Spanish farm. At the time of sampling, the mean age of the animals was 4.7 ± 1.5 years and the mean of parity was 3.2 ± 1.46. On this farm, the animals in the peripartum period (from day 50 before calving to day 30 after calving) were housed in stalls in groups of 20–25 cows. The cows were given free access to water and a nutritionally balanced diet according to their stage (gestation or lactation). Animals were retrospectively selected according to their body condition score (BCS) < 3 at −20 ± 6.91 days relative to calving. A saliva and blood sample were taken from each animal about 13 days before calving (T-13, $n = 12$), within the first 12 h after calving (T0, n = 12), and at 7 (T + 7, n = 12), and 20 (T + 20, $n = 10$) days after calving.

The experimental procedure used for the collection of these samples and the rest of the information from the animals are described in the previous report. White blood cell (WBC) count data and serum Hp values from the above study were also used to analyze their possible correlation with saliva results.

2.2.5. Changes in Hp in lameness

A total of 48 saliva samples from a previous report has been used for the evaluation of changes in salivary Hp in cows with lameness (Contreras-Aguilar et al., 2020). Saliva samples were taken from Holstein-Friesen multiparous cows from a farm in southeastern Spain were included in this experiment. The cows were fed with a total mixed base ration offered once daily *ad libitum*. From - 50 days relative to calving, they received a gestation diet, and once calving the cows received a lactation diet. Water intake was also *ad libitum*. At the time of sampling, all cows were in lactation (from 18 to 207 days in milk). The samples were from the following groups:

(1) Lameness group (L) ($n = 12$). These samples belong to animals with untreated laminitis. All cows showed lameness processes associated with non-infectious lameness (ulcers, white line disease/abscesses, and toe ulcers).
(2) Healthy group (H) (n = 12). These samples belong to healthy animals and were collected at the same time as the previous group.

The experimental procedure used for the collection of these samples, and the rest of the information from the animals was described in the previous report.

2.3. Statistical analysis

The analysis of the method validation (means, SDs, CVs, and linear regression) was performed using routine descriptive statistics procedures (Excel 2019, Microsoft). Statistical analysis and graphs of the results obtained were performed using Graph Pad software (GraphPad Prism, version 9 for Windows, Graph Pad Software Inc., San Die-go, USA).

For both experiments, the normality of the data was assessed using the Shapiro-Wilk test, showing a non-normal distribution. The logarithmic transformation of the data from peripartum experiment was performed, obtaining the logarithmic values. Due to the lack of two missing data in this experiment, these data were analyzed using a mixed-effects analysis and a multiple comparison test. Data from the lameness experiment were analyzed using the Mann-Whitney test which compares ranks of non-parametric data. To determine any significant differences in Hp values obtained in the peripartum experiment, Fisher's test was applied. Results were reported as median and the 25 and 75 percentiles of results were expressed as ng/ml of Hp and represented in box and whiskers plots in Figs. 4 and 5. Significance was set for $p < 0.05$.

The relationship between each salivary sample from the experimental model during peripartum at each time with the WBC count (Wagner et al., 2008) and serum values of Hp were assessed for correlation using Spearman's correlation coefficients.

3. Results

3.1. Analytical validation of the assay

Intra-assay precision showed a mean CV of 4.94% and inter-assay precision of 10.2% (Table 1). Both samples tested (high and low concentration) were linear after serial dilutions showing a coefficient of determination of $R^2 = 0.998$ and $R^2 = 0.986$ respectively (Fig. 3). Furthermore, the mean recovery test was 113% for the high concentration sample and 97.3% for the low concentration sample (Table 2). The LLQ was set at 7.9 ng/ml. The LD could not be calculated since all values obtained were zero (González-Arostegui et al., 2022).

3.2. Changes in salivary Hp during peripartum

Hp concentrations in saliva of cows during peripartum are shown in Fig. 4. Hp values were significantly higher at calving (T0) (mean, 897.7 ng/ml) than at day 13 before calving (T-13) (mean, 249.9 ng/ml) with an increase of 3.6-fold. Hp values at calving (T0) were also significantly higher than at days 7 (T7) (mean, 536.6 ng/ml) and 20 (T20) (median, 304.1 ng/ml) after calving with differences of 1.6-fold and 2.9-fold, respectively. Significant differences between T-13 and T7 were also found (2.1-fold).

3.3. Relationship between salivary Hp and WBC and serum Hp values

No significant correlations were found between both analytes studied, as shown in Table 3.

Table 1
Mean concentration, standard deviation (SD), and an intra- and inter- assay coefficient of variation (CV) for saliva samples with low, medium and high concentration of haptoglobin (Hp) measured with AlphaLISA.

Sample	Intra-assay			Inter-assay		
	Mean (ng/ml)	SD	CV (%)	Mean (ng/ml)	SD	CV (%)
Low Hp	565.4	29.8	5.7	612.5	65.8	10.7
Medium Hp	346.3	22.1	6.4	392.8	33.4	8.5
High Hp	124.4	3.9	3.1	146.3	16.6	11.3

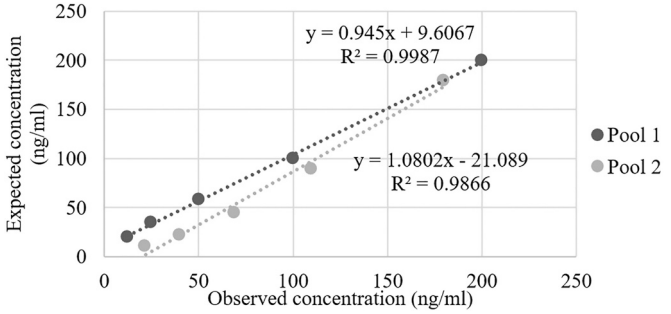

Fig. 3. Regression lines showing haptoglobin (Hp) concentrations (ng/ml) in the AlphaLISA assay for two samples under dilution. Coefficients of determination (R^2) are shown on the figure.

3.4. Changes in salivary Hp in lameness cows

When salivary Hp levels in both groups were analyzed, significant differences were found between the L group (mean, 399.5 ng/ml) and the H group (mean, 165.4 ng/ml) with an increase of 2.4-fold. The results are graphically represented in Fig. 5.

4. Discussion

In this study, an assay for the measurement of Hp in bovine saliva has been developed and validated. This allows the quantification of Hp in saliva of cows, which to the author's knowledge, it has never been described to date. The results of the analytical validation showed intra- and inter-assay imprecision below the recommended 20% (Brunzendorf and Behrens, 2007) and are similar to those previously obtained in other analytes in cows (López-Arjona et al., 2021). This data, together with the high correlation coefficient obtained in the linearity and recovery tests, make this assay valid for the measurement of Hp in bovine saliva samples with high specificity.

As this is the first time that Hp has been measured in bovine saliva and there are no established reference values for this species and sample type to compare, a serum sample of known concentration (1.28 g/l) was used as a calibrator. The Hp values in serum are 1000 times higher compared to those in saliva (Cerón et al., 2008). However, the low LLQ obtained (7.9 ng/ml) is indicative of the high sensitivity of the assay, allowing the detection of salivary Hp levels and better differentiation between healthy and diseased animals. Moreover, AlphaLISA technology has other advantages compared to the enzyme-linked immunosorbent assay, such as shorter incubation time and no need for washing between steps, as well as using a smaller sample volume (5 µl) (López-Martínez et al., 2022).

The measurements during peripartum were made to test whether the assay can detect different salivary Hp concentrations during this stage since significant increases in serum Hp at calving have been observed in other studies, decreasing during the first week postpartum (Arfuso et al., 2022; Humblet et al., 2006). This assay has been able to detect the same pattern in salivary Hp concentrations, although at 1000 times lower levels. Since in this case, we dealt with non-complicated parturitions and healthy cows, this increase in Hp could be related to the inflammation of the uterus during parturition which leads to the release of IL-6 (Arfuso et al., 2022) or by the action of prostaglandin F2α, present at this stage and which has also been shown to activate the synthesis of Hp (Regassa and Noakes, 1999). Increases in salivary Hp of higher magnitude than those found in our report could be related to complications during

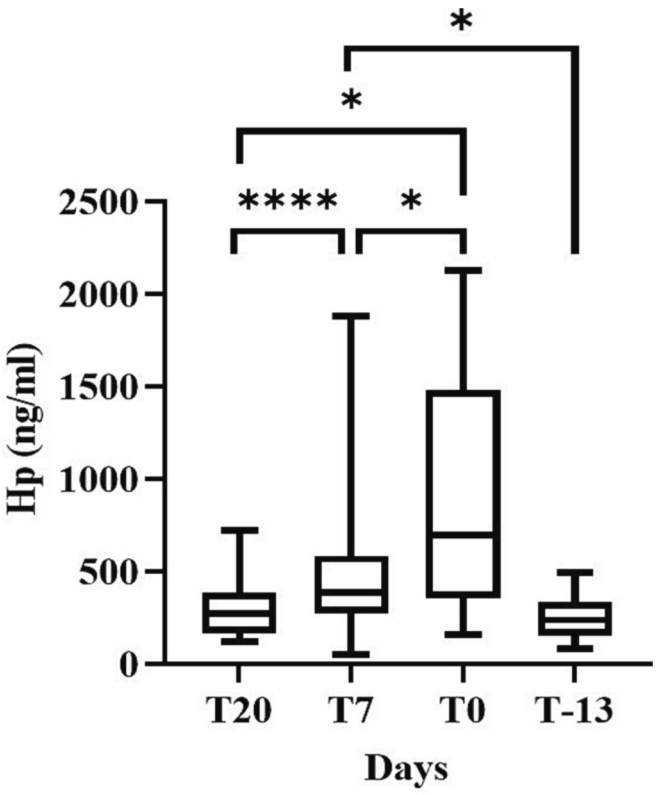

Fig. 4. Haptoglobin (Hp) concentrations (ng/ml) in saliva samples of cows at day 13 before calving (T-13), at the day of calving (T0) and 7 (T7) and 20 (T20) days after calving. Asterisks indicate significant differences (****$p < 0.0001$; *$p < 0.05$).

Table 3
Correlation coefficients between haptoglobin (Hp) in saliva and plasma and between salivary Hp and white blood cell (WBC).

	Spearman r	*p*-value
Saliva - Serum	0.272	0.071
Saliva - WBC	0.135	0.376

Table 2
Recovery of haptoglobin (Hp) in saliva samples.

	Sample		Expected (ng/ml)	Detected (ng/ml)	Recovery (%)
	Serial dilution	Constant dilution			
High concentration	1:4	1:4	222.8	219.9	98.6
	1:8	1:4	175	218.3	124.7
	1:16	1:4	148.6	175.9	118.3
	1:32	1:4	134.2	142.9	106.4
	1:64	1:4	125.9	148.9	118.2
Low concentration	1:4	1:4	91.3	89.1	97.5
	1:8	1:4	59.7	58.9	98.7
	1:16	1:4	44.2	43.2	97.6
	1:32	1:4	34.5	32.4	94.1
	1:64	1:4	27.1	27.2	100.3

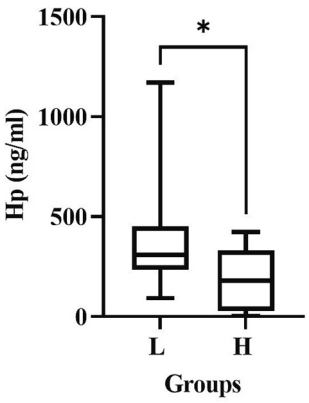

Fig. 5. Haptoglobin (Hp) concentrations (ng/ml) in saliva samples of cows with lameness (L) and in saliva samples of healthy (H) cows at the same time. Asterisks indicate significant differences (* $p < 0.05$).

parturition such as sepsis (Schönfelder et al., 2005; Shin et al., 2018). The cause of the lack of correlation between salivary and plasma Hp values could be due to the different methods used in each case (AlphaLISA technology *vs* automated chemistry analyzer) (Contreras-Aguilar et al., 2021), and also the mechanism leading to the presence of Hp in saliva should be explored (Cerón et al., 2022; Gutiérrez et al., 2012; Lin et al., 2021).

In this study, samples from cows with lameness were also evaluated. A 2.4-fold increase in mean salivary Hp values was observed in lame cows of our study compared to healthy cows. The increase in salivary Hp found in our study would be in line with other studies that reported that serum Hp levels were higher in lame cows than in healthy cows (Bagga et al., 2016; Nazifi et al., 2012; Sun et al., 2015). However, other reports did not find these differences in Hp between lame and healthy cows (Jawor et al., 2008; Kujala et al., 2010).

5. Conclusions

The assay described here can measure in a precise, sensitive, and accurate way the Hp in saliva of cows. In addition, it can detect the increases that occur in inflammatory conditions such as peripartum and lameness. Further studies should be made in a larger population to evaluate the diagnostic ability of this assay to detect inflammation in comparison with other established APPs in serum.

Declaration of Competing Interest

The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript; or in the decision to publish the results.

Acknowledgements

This research was funded by the European Union's Horizon 2020 research and innovation program under grant agreement No. 862919. M. B. has a fellowship of the Séneca Foundation. D.E. was funded by the postdoctoral contract "Generational renewal to promote research" of the University of Murcia. M.L.-A. has a post-doctoral fellowship "Juan de la Cierva Formación" (FJC2021-047105-I) supported by the "Ministerio de Ciencia e Innovación".

References

Andersen, C.B.F., Stødkilde, K., Sæderup, K.L., Kuhlee, A., Raunser, S., Graversen, J.H., Moestrup, S.K., 2017. Haptoglobin. Antioxid. Redox Signal. 26, 814–831. https://doi.org/10.1089/ARS.2016.6793.

Arfuso, F., Minuti, A., Liotta, L., Giannetto, C., Trevisi, E., Piccione, G., Lopreiato, V., 2022. Stress and inflammatory response of cows and their calves during peripartum and early neonatal period. Theriogenology 196. https://doi.org/10.1016/J.THERIOGENOLOGY.2022.11.019.

Bagga, A., Randhawa, S.S., Sharma, S., Bansal, B.K., 2016. Acute phase response in lame crossbred dairy cattle. Vet World 9, 1204. https://doi.org/10.14202/VETWORLD.2016.1204-1208.

Brunzendorf, J., Behrens, R., 2007. How to type test the coefficient of variation of an indication. Radiat. Prot. Dosim. 123, 21–31. https://doi.org/10.1093/RPD/NCL078.

Burnett, T.A., Madureira, A.M.L., Silper, B.F., Nadalin, A., Tahmasbi, A., Veira, D.M., Cerri, R.L.A., 2014. Short communication: factors affecting hair cortisol concentrations in lactating dairy cows. J. Dairy Sci. 97, 7685–7690. https://doi.org/10.3168/JDS.2014-8444.

Caplen, G., Held, S., 2021. Changes in social and feeding behaviors, activity, and salivary serum amyloid a in cows with subclinical mastitis. J. Dairy Sci. 104, 10991–11008. https://doi.org/10.3168/jds.2020-20047.

Ceciliani, F., Ceron, J.J., Eckersall, P.D., Sauerwein, H., 2012. Acute phase proteins in ruminants. J. Proteome. https://doi.org/10.1016/j.jprot.2012.04.004.

Cerón, J.J., Martinez-Subiela, S., Ohno, K., Caldin, M., 2008. A seven-point plan for acute phase protein interpretation in companion animals. Vet. J. 177, 6–7. https://doi.org/10.1016/J.TVJL.2007.12.001.

Cerón, J.J., Contreras-Aguilar, M.D., Escribano, D., Martínez-Miró, S., López-Martínez, M.J., Ortín-Bustillo, A., Franco-Martínez, L., Rubio, C.P., Muñoz-Prieto, A., Tvarijonaviciute, A., López-Arjona, M., Martínez-Subiela, S., Tecles, F., 2022. Basics for the potential use of saliva to evaluate stress, inflammation, immune system, and redox homeostasis in pigs. BMC Vet. Res. 18 https://doi.org/10.1186/S12917-022-03176-W.

Chan, J.P.W., Chu, C.C., Fung, H.P., Chuang, S., Lin, Y.C., Chu, R.M., Lee, S.L., 2004. Serum haptoglobin concentration in cattle. J. Vet. Med. Sci. 66, 43–46. https://doi.org/10.1292/JVMS.66.43.

Contreras-Aguilar, M.D., Monkeviciene, I., Ceron, J.J., Silinskas, I., Vallejo-Mateo, P.J., Tecles, F., Martinez-Subiela, S., Tvarijonaviciute, A., Zelvyte, R., 2019. Biochemical changes in saliva of cows with inflammation: a pilot study. Res. Vet. Sci. 124, 383–386. https://doi.org/10.1016/J.RVSC.2019.04.019.

Contreras-Aguilar, M.D., Vallejo-Mateo, P.J., Želvytė, R., Tecles, F., Rubio, C.P., 2020. Changes in saliva Analytes associated with lameness in cows: a pilot study. Animals (Basel) 10, 1–10. https://doi.org/10.3390/ANI10112078.

Contreras-Aguilar, M.D., Vallejo-Mateo, P.J., Lamy, E., Escribano, D., Cerón, J.J., Tecles, F., Rubio, C.P., 2021. Changes in saliva Analytes in dairy cows during Peripartum: a pilot study. Animals (Basel) 11, 1–10. https://doi.org/10.3390/ANI11030749.

di Masi, A., de Simone, G., Ciaccio, C., D'Orso, S., Coletta, M., Ascenzi, P., 2020. Haptoglobin: from hemoglobin scavenging to human health. Mol. Asp. Med. 73, 100851 https://doi.org/10.1016/J.MAM.2020.100851.

Eckersall, P.D., Rodgers, J., Murray, M., Kennedy, P.G.E., Tomlinson, S., Raper, J., 1999. Haptoglobin, the acute phase response and natural human immunity to trypanosomes. Parasitol. Today 15, 251–252. https://doi.org/10.1016/S0169-4758(99)01459-3.

Escribano, D., Fuentes-Rubio, M., Cerón, J.J., 2012. Validation of an automated chemiluminescent immunoassay for salivary cortisol measurements in pigs. J. Vet. Diagn. Investig. 24, 918–923. https://doi.org/10.1177/1040638712455171.

González-Arostegui, L.G., Muñoz-Prieto, A., Tvarijonaviciute, A., Cerón, J.J., Rubio, C.P., 2022. Measurement of redox biomarkers in the whole blood and red blood cell lysates of dogs. Antioxidants 11. https://doi.org/10.3390/antiox11020424.

Gutiérrez, A.M., Yelamos, J., Pallarés, F.J., Gómez-Laguna, J., Cerón, J.J., 2012. Local identification of porcine haptoglobin in salivary gland and diaphragmatic muscle tissues. Histol. Histopathol. 27, 187–196. https://doi.org/10.14670/HH-27.187.

Humblet, M.F., Guyot, H., Boudry, B., Mbayahi, F., Hanzen, C., Rollin, F., Godeau, J.M., 2006. Relationship between haptoglobin, serum amyloid a, and clinical status in a survey of dairy herds during a 6-month period. Vet. Clin. Pathol. 35, 188–193. https://doi.org/10.1111/J.1939-165X.2006.TB00112.X.

Jawor, P., Steiner, S., Stefaniak, T., Baumgartner, W., Rzasa, A., 2008. Determination of selected acute phase proteins during the treatment of limb diseases in dairy cows. Veterinarni Medicina 53, 173–183. https://doi.org/10.17221/1920-VETMED.

Kujala, M., Orro, T., Soveri, T., 2010. Serum acute phase proteins as a marker of inflammation in dairy cattle with hoof diseases. Vet. Rec. 166, 240–241. https://doi.org/10.1136/VR.B4770.

Levy, A.P., Asleh, R., Blum, S., Levy, N.S., Miller-Lotan, R., Kalet-Litman, S., Anbinder, Y., Lache, O., Nakhoul, F.M., Asaf, R., Farbstein, D., Pollak, M., Soloveichik, Y.Z., Strauss, M., Alshiek, J., Livshits, A., Schwartz, A., Awad, H., Jad, K., Goldenstein, H., 2010. Haptoglobin: basic and clinical aspects. Antioxid. Redox Signal. 12, 293–304. https://doi.org/10.1089/ARS.2009.2793.

Lin, G.C., Küng, E., Smajlhodzic, M., Domazet, S., Friedl, H.P., Angerer, J., Wisgrill, L., Berger, A., Bingle, L., Peham, J.R., Neuhaus, W., 2021. Directed transport of CRP across in vitro models of the blood-saliva barrier strengthens the feasibility of salivary CRP as biomarker for neonatal Sepsis. Pharmaceutics 13, 256. https://doi.org/10.3390/PHARMACEUTICS13020256.

López-Arjona, M., Tecles, F., Mateo, S., Contreras-Aguilar, M.D., Martínez-Miró, S., Cerón, J.J., Martínez-Subiela, S., 2020. Measurement of cortisol, cortisone and 11β-hydroxysteroid dehydrogenase type 2 activity in hair of sows during different phases of the reproductive cycle. Vet. J. 259–260 https://doi.org/10.1016/j.tvjl.2020.105458.

López-Arjona, M., Mainau, E., Navarro, E., Contreras-Aguilar, M.D., Escribano, D., Mateo, S., Manteca, X., Cerón, J.J., Martínez-Subiela, S., 2021. Oxytocin in bovine saliva: validation of two assays and changes in parturition and at weaning. BMC Vet. Res. 17 https://doi.org/10.1186/S12917-021-02838-5.

López-Martínez, M.J., Escribano, D., Martínez-Miró, S., Ramis, G., Manzanilla, E.G., Tecles, F., Martínez-Subiela, S., Cerón, J.J., 2022. Measurement of procalcitonin in saliva of pigs: a pilot study. BMC Vet. Res. 18, 1–9. https://doi.org/10.1186/S12917-022-03240-5/FIGURES/5.

Lu, J., Wang, Y., Yan, M., Feng, P., Yuan, L., Cai, Y., Xia, X., Liu, M., Luo, J., Li, L., 2016. High serum haptoglobin level is associated with tumor progression and predicts poor prognosis in non-small cell lung cancer. Oncotarget 7, 41758. https://doi.org/10.18632/ONCOTARGET.9676.

Marchand, A., Galen, R.S., van Lente, F., 1980. The predictive value of serum haptoglobin in hemolytic disease. JAMA 243, 1909–1911. https://doi.org/10.1001/jama.1980.03300450023014.

Naryzhny, S.N., Legina, O.K., 2021. Haptoglobin as a biomarker. Biomeditsinskaya khimiya 67, 105–118. https://doi.org/10.18097/PBMC20216702105.

Nazifi, S., Esmailnezhad, Z., Haghkhah, M., Ghadirian, S., Mirzaei, A., 2012. Acute phase response in lame cattle with interdigital dermatitis. World J. Microbiol. Biotechnol. 28, 1791–1796. https://doi.org/10.1007/S11274-011-0995-9.

Nirala, N.R., Harel, Y., Lellouche, J.P., Shtenberg, G., 2020. Ultrasensitive haptoglobin biomarker detection based on amplified chemiluminescence of magnetite nanoparticles. J Nanobiotechnology 18, 6. https://doi.org/10.1186/S12951-019-0569-9.

Quaye, I.K., 2008. Haptoglobin, inflammation and disease. Trans. R. Soc. Trop. Med. Hyg. https://doi.org/10.1016/j.trstmh.2008.04.010.

Regassa, F., Noakes, D.E., 1999. Acute phase protein response of ewes and the release of PGFM in relation to uterine involution and the presence of intrauterine bacteria. Vet. Rec. 144, 502–506. https://doi.org/10.1136/VR.144.18.502.

Schönfelder, A., Schrödl, W., Krueger, M., Richter, A., Sobiraj, A., 2005. The change of acute phase protein haptoglobin in cattle during spontaneous labor and caesarean section with or without torsio uteri intrapartum. Berl. Munch. Tierarztl. Wochenschr. 118, 240–246.

Shih, A.W.Y., Mcfarlane, A., Verhovsek, M., 2014. Haptoglobin testing in hemolysis: measurement and interpretation. Am. J. Hematol. 89, 443–447. https://doi.org/10.1002/AJH.23623.

Shin, D.H., Jeong, J.K., Choi, I.S., Moon, S.H., Lee, S.C., Kang, H.G., Park, S.B., Kim, I.H., 2018. Associations between serum haptoglobin concentration and peri- and postpartum disorders, milk yield, and reproductive performance in dairy cows. Livest. Sci. 213, 14–18. https://doi.org/10.1016/J.LIVSCI.2018.04.015.

Shu, H., Kang, X., Guo, K., Li, S., Li, M., Sun, L., Gan, L., Liu, Y., Qin, X., 2010. Diagnostic value of serum haptoglobin protein as hepatocellular carcinoma candidate marker complementary to α fetoprotein. Oncol. Rep. 24, 1271–1276. https://doi.org/10.3892/OR_00000982.

Simões, P.B.A., Campbell, M., Viora, L., Gibbons, J., Geraghty, T.E., Eckersall, P.D., Zadoks, R.N., 2018. Pilot study into milk haptoglobin as an indicator of udder health in heifers after calving. Res. Vet. Sci. 116, 83–87. https://doi.org/10.1016/J.RVSC.2017.05.024.

Simon, A., Schneider, N., Gillery, P., Oudart, J.B., 2020. Phénotypes d'haptoglobine : impacts cliniques et biologiques. Ann Biol Clin (Paris) 78, 493–498. https://doi.org/10.1684/ABC.2020.1590.

Smith, B.I., Kauffold, J., Sherman, L., 2010. Serum haptoglobin concentrations in dairy cattle with lameness due to claw disorders. Vet. J. 186, 162–165. https://doi.org/10.1016/J.TVJL.2009.08.012.

Sun, D., Li, C., Gu, C., Chen, J., Qu, Y., Wang, X., Gao, J., Wei, S., Wang, J., Wu, R., Guo, D., 2015. Analysis of mineral elements, metabolism, and inflammation indexes in the plasma of dairy cows suffering from different degrees of lameness. Biol. Trace Elem. Res. 168, 372–379. https://doi.org/10.1007/S12011-015-0380-X.

Tvarijonaviciute, A., Martinez-Subiela, S., Lopez Jornet, P., Lamy, E., 2020. Saliva in Health and Disease The Present and Future of a Unique Sample for Diagnosis. https://doi.org/10.1007/978-3-030-37681-9.

Wagner, S.A., Schimek, D.E., Cheng, F.C., 2008. Body temperature and white blood cell count in postpartum dairy cows. Bov Pract (Stillwater) 18–26. https://doi.org/10.21423/BOVINE-VOL42NO1P18-26.

Wollowski, L., Heuwieser, W., Kossatz, A., Addis, M.F., Puggioni, G.M.G., Meriaux, L., Bertulat, S., 2021. The value of the biomarkers cathelicidin, milk amyloid a, and haptoglobin to diagnose and classify clinical and subclinical mastitis. J. Dairy Sci. 104, 2106–2122. https://doi.org/10.3168/JDS.2020-18539.