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THE ACUTE-PHASE AND HEMOSTATIC RESPONSE IN DROMEDARY CAMELS (*CAMELUS DROMEDARIUS*)

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Abstract: Acute-phase reactants indicate inflammation and are increasingly used in veterinary medicine to indicate and to monitor progression of disease. Hemostasis and inflammation have interconnected pathophysiologic pathways and influence each other on different levels. This study established observed normal ranges for acute-phase reactants and for coagulation and thromboelastographic (TEG) parameters in 49 dromedary camels (*Camelus dromedarius*) and assessed the response to chronic and acute inflammation. Chronically infected animals suffering from lymph abscessation due to *Corynebacterium* spp. had significantly higher concentrations of the acute-phase reactants haptoglobin ($P < 0.005$) and fibrinogen ($P < 0.013$) and an increased clot strength characterized by an increase of the TEG parameters MA ($P < 0.039$), representing the maximum amplitude of the clot strengths, and G, the global clot strength ($P < 0.022$), compared to healthy animals. When the acute-phase and hemostatic responses of 10 males receiving a gonadotropin-releasing hormone vaccine and of 9 males that were surgically castrated over 7 days were studied, haptoglobin proved to be a minor positive acute-phase protein, with moderate levels in healthy animals. It increased significantly after both vaccination and castration and remained elevated 7 days postinsult. The negative reactant iron significantly decreased over the 7-day period after castration, whereas a similar decrease following vaccination lasted less than 3 days. Fibrinogen reacted as a positive, minor reactant, with a significant increase and a peak on days 3–5, with higher values seen after castration. Prothrombin time showed a slight shortening at days 5–7, and the TEG parameters MA and G showed significantly increased values, similar to fibrinogen. The acute-phase protein serum amyloid A showed poor repeatability, suggesting that the assay was not reliable.

Key words: Coagulation, fibrinogen, haptoglobin, inflammation, iron, thromboelastography.

INTRODUCTION

Blood levels of a variety of parameters change in response to ongoing inflammation, and can therefore be measured to indicate inflammation or to monitor progression of disease. The most common reactants are iron, total protein (TP), and acute-phase proteins (APPs).[12,23,31] For example, blood iron levels rapidly decrease in response to inflammation as part of the body's nonspecific resistance to bacterial infection and proliferation.[5] The production of immunoglobulins can significantly increase after an inflammatory stimulus and therefore elevate TP in the blood, making TP a useful diagnostic indicator of inflammation.[21]

APPs are a group of plasma proteins that have been shown to change their blood levels by more than 25% in response to various inflammatory and noninflammatory conditions, such as trauma, infection, stress, and neoplasia.[9,22] They are increasingly used in veterinary medicine for diagnostic purposes. Major APPs display an immediate and more than 10-fold response following an inflammatory stimulus and normalize again within a short period of time. In contrast, minor APPs exhibit a smaller and more graduated response that usually lasts longer.[22] The serum concentration of APPs can either increase (positive APPs) or decrease (negative APPs). Fibrinogen, serum amyloid A (SAA), and haptoglobin are generally considered positive APPs, whereas albumin is a negative APP.[8,26]

The pathophysiologic pathways of inflammation and hemostasis are interconnected, and cross talk between the two pathways can happen at different levels: through production of cytokines

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(eg, interleukins 1 and 6), by protein synthesis (fibrinogen and factor VIII), or through direct interaction between cells (platelets, leukocytes, and endothelial cells).[3,24,25] The hemostatic system can be evaluated by measuring coagulation parameters such as activated prothrombin time (APTT), prothrombin time (PT), and fibrinogen in blood samples. Additionally, the global hemostatic ability can be evaluated by thromboelastography (TEG).

The acute-phase response of dromedary camels (*Camelus dromedarius*) has attracted some attention, and it is generally accepted that haptoglobin, fibrinogen, and SAA are positive APPs, whereas albumin is a negative APP in this species.[2,13,14,16,33] However, there is a lack of prospective studies following reactants over time after an inflammatory stimulus. This knowledge is essential to interpret the timing of the inflammatory process, to accurately monitor the progress of disease, and to evaluate treatment response. Further, the understanding of the hemostatic parameters in camels is limited,[17,18] and the potential relation between the inflammatory and hemostatic response undescribed. The objective of this study was therefore to characterize the development of hemostatic parameters and acute-phase reactants over 7 days after an acute onset of trauma and to assess the difference in these parameters between healthy and chronically infected camels. Additional aims were to establish normal ranges of hemostatic parameters and acute-phase reactants in healthy dromedary camels.

MATERIALS AND METHODS

Animals and sampling protocols

Forty-nine dromedary camels housed at Oasis Park, Fuerteventura, Spain were included in the study following permission from the institutional animal care and use committee. The study consisted of two parts: a cross-sectional study and a prospective experiment. The cross-sectional study included 10 adult females and 19 adult males deemed healthy based on their medical history, clinical appearance, and a hematologic evaluation, and 10 male and 10 female adult dromedary camels chronically infected with *Corynebacterium* sp. diagnosed as the cause of disseminated lymph node abscessation via culture prior to the study. The infected camels were housed separately from the other animals, and they all exhibited chronic purulent lymph node abscessation at the time of sampling.

Samples were collected under manual restraint with the exception of 15 adult healthy males, from which blood was collected under α-2 agonist–based sedation or anesthesia.

The prospective part of the study involved the 19 adult healthy males. These males were split into two groups: 10 males were chemically contracepted with a gonadotropin-releasing hormone (GnRH) vaccine, and 9 males were surgically castrated. Sedated or anesthetized animals were fasted for 48 hr and water was restricted for 24 hr prior to the intervention. Following sedation or manual restraint, the 10 animals were vaccinated with a commercially available GnRH vaccine (Improvac, Zoetis Belux, 1930 Zaventem, Belgium; 800 μg im sid), and a fine-needle biopsy sample was collected from one or both testes using a 21-ga needle. The remaining nine healthy male camels were surgically castrated under general anesthesia. The anesthesia was induced either with hand injection under physical restraint or using remote injection. Following infiltration of lidocaine (Lidocaina 20 mg/ml injectable solution, Laboratorios Normon S.A., 28760 Tres Cantos, Madrid, Spain; 100 mg) into each spermatic cord, surgery consisted of one longitudinal incision of the scrotum and a consecutive forward displacement of the testes within the intact vaginal tunics. Two absorbable ligatures (Surgicryl PGA, SMI, 4780 St. Vith, Belgium) were placed on each spermatic cord and the testes were cut distally to the ligatures. The surgical incision was left open to heal by secondary intention. Benzyl penicillin benzathine and benzyl penicillin procaine (Benzatard, Laboratorias SYVA S.A.U., 24010 León, Spain; 6.000 IU/kg im, sid) and vitamin E and selenium (Esveex, S.P. Veterinaria S.A., 43330 Riudoms, Spain; 1.5 g vitamin E and 5 mg selenium sc sid) were administered once. No anti-inflammatory drugs were administered after the castration, because it has been demonstrated that this could alter the response of the reactant haptoglobin in cattle.[30] One of the vaccinated animals showed scrotal swelling the day after vaccination and another regurgitated slightly during sedation. In one castrated animal, pronounced scrotal inflammation was observed 72 hr after the surgery. These three animals received injections with Benzatard 6.000 UI/kg im sid every 72 hr three times consecutively. In the castrated and vaccinated camels, blood was collected on days 0, 2, 3, 5, and 7 after the intervention.

All blood samples were collected from the jugular vein using a butterfly needle and a

vacutainer system (Becton-Dickinson UK Limited, RG41 5TS, Berkshire, United Kingdom), filling a plain tube (Becton-Dickinson UK Limited) first followed by a citrated tube (Becton-Dickinson UK Limited). At the first day of sampling of each individual, an additional EDTA tube (Becton-Dickinson UK Limited) was filled as the last tube. The collected blood was stored cool for a maximum of 14 hr before being centrifuged at 1,370 *g* for 10 min. Following centrifugation, citrated plasma and serum were transferred to cryovials (Nunc CryoTube Vials, VWR International LLC, Radnor, Pennsylvania 19087, USA) and stored at −20°C for a maximum of 2 wk, after which samples were stored at −80°C until analysis. On the day of collection, a whole blood count and differential of the camels were performed on EDTA blood using an automatic analyzer (VetScan HM5, Abaxis, Union City, California 94587, USA). The interpretation of the results was based on ZIMS references.[32]

Laboratory assays

The plasma and serum samples were analyzed at the Veterinary Diagnostic Laboratory at the University of Copenhagen, Denmark, 4 mo after sampling. SAA, haptoglobin, iron, albumin, and TP were measured in serum. Citrate plasma was used for TEG analysis, to measure fibrinogen and the coagulation parameters APTT and PT. APP, iron, and TP analyses were performed in duplicate within one analytic run. SAA and serum haptoglobin were determined by a solid-phase sandwich enzyme-linked immunosorbent assay kit (Tridelta Development Ltd., W 23 Maynooth, County Kildare, Ireland). The measurements of iron, albumin, and TP were performed using an automated clinical chemistry analyzer (ADVIA 1800 Chemistry System, Siemens Healthcare Diagnostics, 2750 Ballerup, Denmark) following the manufacturer's instructions. The parameters APTT, PT, and fibrinogen were measured using an automated coagulometric analyzer (ACL Top500, Instrumentation Laboratory, 3450 Allerød, Denmark) according to the manufacturer's instructions. Fibrinogen and PT were measured with a high-sensitivity thromboplastin agent (HemosIL, RecombiPlasTin 2G, Instrumentation Laboratory) and APTT with a high-quality synthetic phospholipid reagent (HemosIL, SynthAFax, Instrumentation Laboratory). The analyses of TEG were performed as previously described by Wiinberg and Jensen.[36] A computerized thromboelastograph (TEG 5000 Hemostasis Analyzer System, Haemonetics, Braintree, Massachusetts 02184-9114, USA) with continuous data acquisition was used. Each sample was activated using a solution of recombinant human tissue factor (Innovin, Dade Behring, Siemens Healthcare Diagnostics) at a final dilution of 1 : 50,000. The parameters chosen for further evaluation were reaction time (R), representing the time until the first evidence of clot formation; angle (α), representing the speed of clot formation; the maximum amplitude of the strength of the clot (MA); and the global clot strength (G), which describes the overall strength of the clot that is formed.[37]

Statistical analysis

Statistical analysis was performed using GraphPad Prism version 7 for Windows (GraphPad Software, Inc. La Jolla, California 92037, USA). The mean of duplicate measurements was calculated and used for further analyses. The average coefficient of variation between duplicates was calculated, and intra-assay values less than 10% were regarded as acceptable.

Normality of data was tested using the D'Agostino and Pearson normality test, or alternatively the Shapiro-Wilk normality test for smaller sample sizes. Observed intervals for clinically healthy animals, including the median, minimum, maximum, and 25th and 75th percentiles, were calculated for all analyzed parameters. Results from healthy camels were compared with those from abscessed camels using an unpaired *t*-test if the data were normally distributed, or alternatively a Mann-Whitney test if data were not normal.

For vaccinated and surgically castrated camels, the resting values (day 0) were compared to those from days 1, 3, 5 and 7 after the intervention. Normally distributed data were compared with a one-way analysis of variance, followed by a Dunnett multiple comparison test. Nonnormal data were compared with a Friedman test, followed by a Dunn multiple comparison test. Differences were considered significant at values of $P < 0.05$.

RESULTS

Observed normal ranges of the 29 clinically healthy animals, including the median, minimum, maximum, and 25th and 75th percentiles, are given in Table 1. None of the measured reactants in healthy camels differed between sexes.

Camels infected with *Corynebacterium* spp. had significantly higher concentrations of haptoglobin ($P < 0.005$) and fibrinogen ($P < 0.013$) compared

Table 1. Observed normal ranges, including the calculated intra-assay coefficient of the variability (IACV), of the acute-phase reactants albumin, total protein (TP), iron, haptoglobin, serum amyloid A (SAA), and fibrinogen and observed normal ranges of the hemostatic parameters activated prothrombin time (APTT) and prothrombin time (PT) and the thromboelastographic parameters reaction time (R), angle (α), maximum amplitude of clot strength (MA), and global clot strength (G) in 29 clinically healthy adult dromedary camels *(Camelus dromedarius*; 10 females and 19 males).

Parameter	Median	Minimum	Maximum	25th–75th percentile	IACV (%)
Albumin (g/L)	36.19	26.17	45.58	31.82–39.21	1.5
TP (g/L)	63.38	48.71	80.91	55.22–67.99	1.1
Iron (μM/L)	15.1	5.75	23.2	12.5–17.13	2.9
Haptoglobin (mg/L)	481.8	233.8	2,114	383.4–701.1	2.1
SAA (ng/ml)	17.95	2.4	118.1	12.75–37.59	45.2
Fibrinogen (g/L)	3.98	2.93	5.83	3.47–4.82	2.1
APTT (sec)	32.4	28.8	45.4	29.85–35.2	
PT (sec)	14.9	12.2	19.1	14–16.7	
R (min)	13.8	3.8	92.1	8.25–18.6	
α (°)	20.65	1.8	71.3	17.48–39.05	
MA (mm)	20.2	8.3	30.4	15.7–22.38	
G (kilodynes/cm^2)	1.3	0.5	2.2	0.9–1.48	

to healthy animals (Fig. 1). A significant difference was also seen for the hemostatic TEG parameters MA ($P < 0.039$) and G ($P < 0.022$). Abscessed camels showed significantly higher white blood cell counts (healthy median = 10.24 $\times$ 10^9/L; abscessed median = 13.56 $\times$ 10^9/L; $P < 0.001$) and platelet counts with a large overlap within the two groups (healthy median = 82 $\times$ 10^9/L; abscessed median = 117 $\times$ 10^9/L; $P < 0.024$); the hematocrit did not change significantly. The serum concentrations of albumin, TP, iron, and SAA did not show significant differences, nor did the hemostatic parameters APTT and PT or the TEG parameters R and α. In abscessed camels, albumin was significantly higher in females than in males (females, median = 36.76 g/L; males, median = 33.68 g/L; $P < 0.031$). Remaining tested reactants did not differ between infected males and females.

In the prospective study, haptoglobin increased significantly in all castrated animals on day 3 ($P < 0.027$), peaking on day 5 ($P < 0.001$) and remaining significantly increased through day 7 ($P < 0.001$) (Fig. 2). The vaccinated camels showed an increase of haptoglobin on day 1 ($P < 0.008$), and the values remained significantly increased throughout the 7-day period ($P < 0.011$), with a peak on day 3 and a slight decrease on days 5 and 7 (Fig. 2).

Following castration, fibrinogen was significantly increased at all time points ($P < 0.001$), peaking on day 5 (Fig. 2). Fibrinogen in vaccinated camels was mildly but significantly elevated from day 3 through day 7 ($P < 0.001$) (Fig. 2). SAA showed no significant difference over 7 days in either the castrated or the vaccinated group.

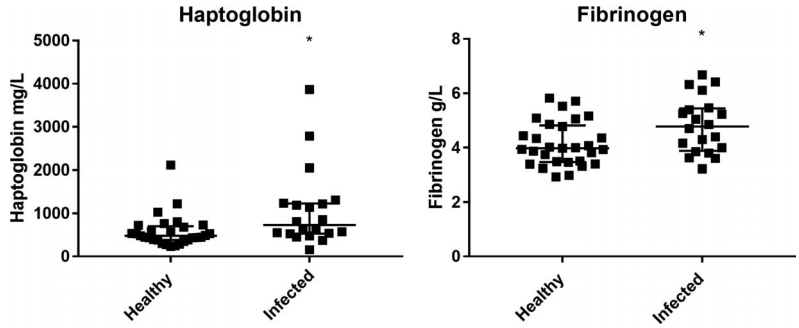

Figure 1. Median ± interquartile ranges of the serum concentrations of the acute-phase reactants haptoglobin and fibrinogen in comparison between healthy and chronically infected dromedary camels (*Camelus dromedarius*). Significant differences ($P < 0.05$) are indicated with an asterisk (*).

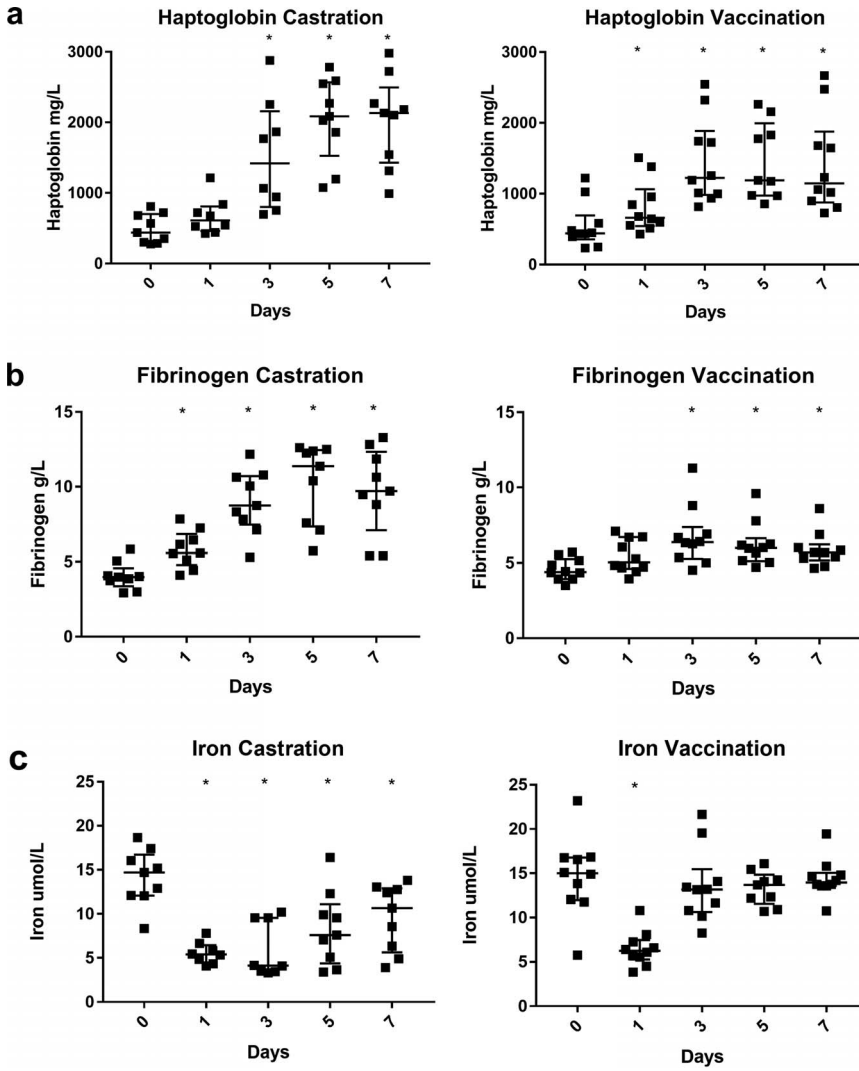

Figure 2. Median ± interquartile ranges of the serum concentration of the acute-phase reactants (**a**) haptoglobin, (**b**) fibrinogen, and (**c**) iron over the period of 1 wk in castrated and vaccinated dromedary camels (*Camelus dromedarius*). Significant differences ($P < 0.05$) are indicated with an asterisk (*).

Iron was significantly decreased in the castrated animals throughout the whole study period ($P < 0.001$). The lowest value was detected on day 3 ($P < 0.001$), after which values increased but remained significantly lower than on day 0 (Fig. 2). The iron values of the vaccinated camels decreased only on day 1 ($P < 0.002$), to about 50% of original values (Fig. 2).

Over the 7-day period following castration, albumin increased significantly on day 1 ($P < 0.014$, median = 35.4 g/L) and then decreased towards the baseline. TP was significantly elevated on days 1 ($P < 0.002$, median = 61.8 g/L) and 3 ($P < 0.044$, median = 58.0 g/L) after castration. The vaccinated animals did not show changes in albumin or TP.

PT was significantly shortened on days 7 and 5 in the castrated ($P = 0.004$) and vaccinated animals ($P = 0.046$), respectively (Fig. 3). Except on days 1 and 7 in the group of vaccinated camels, the TEG values MA and G were significantly increased over the 7-day period for both groups. Both parameters peaked on day 7 in surgically castrated animals (MA, $P < 0.001$; G, $P < 0.001$) and both parameters peaked on day 3 in vaccinated animals (MA, $P < 0.001$; G, $P <$

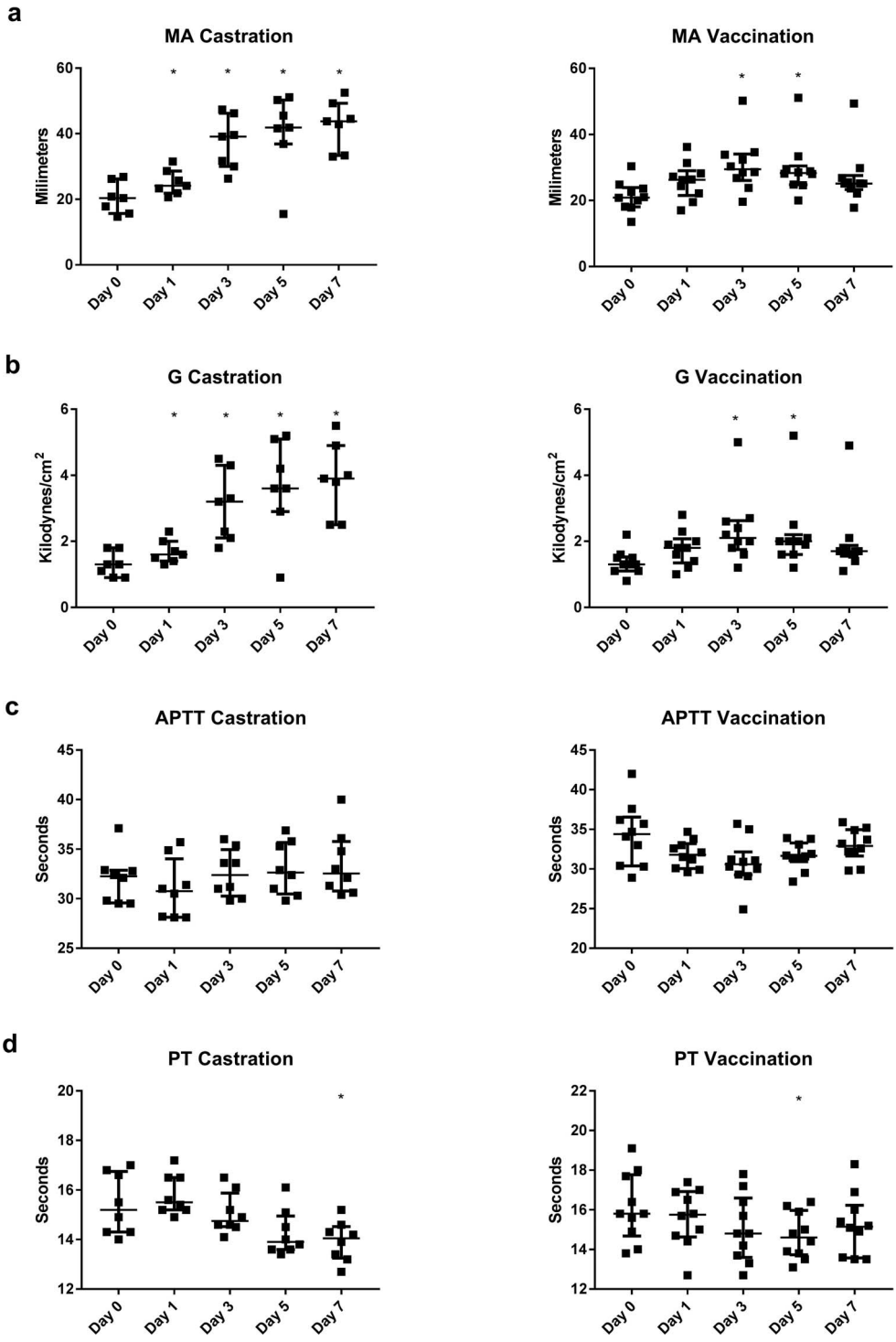

Figure 3. Median ± interquartile ranges of thromboelastography parameters maximum amplitude of clot strength (MA) (**a**) and overall global strength of the clot (G) (**b**) and coagulation parameters activated partial thromboplastin time (APTT) (**c**) and prothrombin time (PT) (**d**) in castrated and vaccinated dromedary camels (*Camelus dromedarius*) over the period of 1 wk. Significant differences ($P < 0.05$) are indicated with an asterisk (*).

0.001) (Fig. 3). The remaining parameters, R, APTT, and α, showed no difference over time.

DISCUSSION

In the prospective study, haptoglobin and fibrinogen proved to act as positive APPs in dromedary camels, increasing significantly after acute trauma. Both remained elevated 7 days postinsult, thus showing a prolonged response, indicating that they have a long half-life in this species. Haptoglobin is a major APP in cattle, showing negligible circulating levels in the serum of healthy animals.[8,11] However, in this study, as in several previous reports,[4,13,27,28] haptoglobin was measurable in healthy animals. In this study, chronically infected animals had significantly higher haptoglobin values than healthy animals, which was also previously demonstrated following both infectious and noninfectious stimuli measured at a single time point.[2,13–16,33] Overall, the findings indicate that haptoglobin acts as a minor APP in dromedary camels, in contrast to cattle.[22]

In this prospective study, a single inflammatory stimulus led to an increase in fibrinogen, peaking on days 3–5. A more pronounced increase was seen following surgery than because of chemical immunostimulation. Dromedary camels with chronic inflammation showed significantly higher fibrinogen values compared to healthy camels. This confirms previous reports of elevated fibrinogen in dromedary camels affected by infectious as well as noninfectious conditions.[13–16] In summary, fibrinogen was shown to be a positive, minor APP in dromedary camels because of the prolonged elevation, not reaching initial baseline values even 7 days postintervention.

Iron levels were not significantly altered in chronically infected camels. Horses with systemic acute and chronic inflammation have significantly lower iron concentrations than those with local inflammation.[6] Dromedary camels might be able to mount a more local inflammatory reaction, even though the lymph node abscessation is marked. One study measuring iron levels in camels subclinically infected with theilerioses did not show a significant iron decrease.[38] In the present study, a week-long and significant decrease in iron was seen upon surgical castration, and a much shorter but significant decrease of less than 3 days following vaccination. The longer response in the castrated camels is probably due to the more intensive systemic inflammatory stimulus inflicted by surgery. Severe blood loss could also explain low iron values, but this was not observed in any of these cases.

An increase of varying magnitude in SAA has been observed in a variety of conditions.[2,13–16,33] The observed baseline value for SAA in healthy dromedary camels in this study (median = 17.95 ng/ml) is about 500-fold lower than most previously reported normal values, but slightly higher than seen in racing camels (mean = 0.7 ng/ml).[2,13–16,33] There was no significant difference in SAA among the different groups of camels in this study. The measured average coefficient of variation between the measured duplicates of 45% suggests that the Tridelta assay may not be reliable in this species, whereas the other assays used in this study show an average coefficient of variation lower than 3%, suggesting an excellent repeatability. The literature provides very little information on the dilutions required for producing valid results; therefore, SAA data on dromedary camels should be interpreted with care.

Albumin showed no significant decrease either in chronically infected animals or in the prospective study. In horses and cattle, a decrease in albumin due to inflammation takes several weeks to develop, suggesting that a longer study period would be necessary to detect if albumin is acting as a negative reactant in camels.[20] TP showed no difference in chronically infected or vaccinated animals. With the exception of significantly lower TP in calves with pneumonia,[14] this result is supported by other publications including both infectious and noninfectious conditions.[2,4,15,16,38] Albumin significantly increased 1 day and TP 1–3 days after surgery. This could theoretically be attributed to a mild dehydration due to water restriction prior to anesthesia, which is interesting because camels are known to be resistant to dehydration. One study showed a significant change in TP and albumin after water restriction only after 5 and 10 days, respectively.[10]

In healthy dromedary camels, the observed normal ranges of the coagulation parameters APTT and PT were established and found to be similar in chronically infected animals. The established ranges for APTT and PT in healthy individuals were slightly different from those in previous reports,[17,18] possibly because of different reagents and instruments used. In the prospective study, PT showed a slight shortening at days 7 and 5 in the castrated and vaccinated groups respectively. This substantiates the connection between the inflammatory and hemostatic systems because the same days showed marked reaction in the inflammatory system. However, in the surgically

castrated animals, fibrinogen was markedly increased during the entire study period. The lack of observed changes in APTT in either group together with the lack of change in the PT concomitant with fibrinogen may be due to higher fibrinogen assay sensitivity compared to APTT and PT. The TEG parameters support the findings of the single coagulation parameters because of no significant changes in the parameters evaluating the initiation of clot formation. Comparing healthy versus chronically infected animals, an increase of the values MA and G is present. These values represent the strength of the formed clot. These findings are supported by significant changes in fibrinogen in the same group of animals, as fibrinogen is an important substrate in clot formation. In the prospective study, MA and G showed significant changes, similar to fibrinogen.

Both general anesthesia and manual restraint of animals can induce stress. In horses, a stress-induced increase in APPs has been documented,[7] which can be speculated to have influence in this study as well. A study addressing the impact of racing demonstrated a significant increase in haptoglobin and SAA values,[33] whereas road transport in camels failed to demonstrate increases in haptoglobin, fibrinogen, SAA, TP, and albumin.[4]

Generally, the results of this study suggest that sex has no effect on the parameters studied, which is in line with previous findings.[4] However, albumin was slightly but statistically significantly higher in females suffering from chronic abscesses, which cannot be readily explained.

After sampling, the samples were frozen within several hours; however, the slight differences in temperature and duration in sample storage before freezing due to field conditions and the slightly different storage times at –20°C could have influenced the analyses. SAA levels can be affected by storage temperature and duration,[34] as can TP and albumin.[1] On the other hand, in humans, fibrinogen is proven to remain stable through repeated freeze–thaw cycles and for 24 hr of storage at either room temperature or 4°C.[29] Further, haptoglobin and iron remain stable at –20°C after 1 yr of storage.[19] If a degradation of the analyzed parameters was present in this study because of the handling process, it was probably linear and unlikely to affect conclusions. However, it could lead to reporting lower than actual values. The same could be the case for the hemostatic parameters, which can be affected by storage;[35,39] however, the influence is assessed to be minimal, because the samples were overall treated very similarly.

In summary, this study has established observed normal ranges for acute-phase reactants and hemostatic parameters in healthy dromedary camels. It demonstrates that the APPs haptoglobin and fibrinogen and the TEG hemostatic parameters MA and G are increased in chronically infected camels, and concludes that haptoglobin and fibrinogen are minor positive APPs that remain elevated more than 1 wk after an inflammatory stimulus. Iron acts as a negative reactant in an acute onset of inflammation. This knowledge can be used to evaluate the level of an inflammatory reaction, to adapt treatment regimes, and to monitor ongoing inflammatory or hemostatic disorders in dromedary camels.

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