

Blood coagulation parameters in fallow deer (*Dama dama*)

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ABSTRACT: There are frequent reports from around the world of wild animals being poisoned with anticoagulants. Granulated baits can result in primary or secondary poisoning of non-target animals. Moreover, there are several diseases including infections that influence haemostasis in wild animals. The present study focused on fallow deer (*Dama dama*) for which insufficient data on physiological values of coagulation parameters are available. Six parameters of blood coagulation were established in clinically normal fallow deer from a game enclosure in North Moravia (Czech Republic). The fibrinogen content of 1.94 g/l is in agreement with the results obtained by other authors. Factor VIII and IX concentrations amounted to 198.42% and 169.91% of human concentration of these parameters in blood. These have never before been measured for fallow deer, but most animal species have concentrations of these factors higher than humans. Prothrombin time (PT), average activated partial thromboplastin time (APTT), and thrombin time (TT) were assessed as 20.99 s, 33.76 s, and 24.78 s, respectively. Prothrombin time assessed in the present study was longer compared to available data, while APTT is in agreement with the previous data. Thrombin time value is a new piece of information and is comparable with TT values obtained in other ruminants. The possible explanation for the prolonged PT may be the stress associated with yarding and handling the animals which is reported to cause haemorrhages or changes in haemostatic parameters in deer. Interestingly, males had significantly longer clotting times compared to females.

Keywords: prothrombin time; thrombin time; APTT; fibrinogen; factor VIII; factor IX; clotting time; haemostasis

Fallow deer (*Dama dama*), a non-native species in the Czech Republic, was widely introduced into Europe in recent times (Nowak and Paradiso, 1983). At present, it is found worldwide and is kept in enclosures and deer farms, while wild populations also exist. Annual bag records ranging from about 8000 to 13 000 in the last decade in the Czech Republic attest to its increasing importance as a popular game deer.

As in other animal species, the recognition and intravital diagnosis of diseases and health problems in fallow deer requires a thorough knowledge of an array of reference parameters (Vengust et al., 2002; Pikula et al., 2007). Normal coagulation parameters are necessary for evaluating and understanding the clinical manifestation of haemorrhagic diseases

such as haemorrhagic septicaemia, haemorrhagic enteropathy of yersiniosis, epizootic haemorrhagic disease, malignant catarrhal fever, bluetongue, and mucosal disease (Debbie and Abelseth, 1971; Sutherland et al., 1985; Eriksen et al., 1999; Haigh et al., 2002; Mackintosh et al., 2002). Other common causes of haemorrhage in wildlife include trauma and anticoagulant poisoning (Berny et al., 1997; Stone et al., 1999; Beklova et al., 2007; Valchev et al., 2008). Clotting factor deficiencies can also result from chronic liver and wasting diseases (Green, 2010). To the best of our knowledge, there is only one paper dealing with blood coagulation parameters in clinically normal intensively farmed fallow deer (Sutherland et al., 1985). As this previous study was based on only 10 examined specimens,

Supported by the Internal Grant Agency of the University of Veterinary and Pharmaceutical Sciences, Brno, Czech Republic (Grant No. 204/2009/FVHE).

the objective of the present study was to determine reference values of prothrombin time (Quick test), APTT, thrombin time, fibrinogen content and factor VIII and IX content in the blood plasma of a greater collection of fallow deer from a large game enclosure.

MATERIAL AND METHODS

Blood samples from 34 individuals were obtained between autumn 2009 and spring 2010. Animals involved in this study came from a game enclosure in North Moravia (Czech Republic) and were caught for planned transportation to a new location. They were six months to five years old, with weights ranging from about 20 to 60 kg. A total of 19 females and 15 males were used in the experiment. Animals were physically restrained for blood collection and at the time of sampling all fallow deer appeared healthy and in good nutritional state. Sampling of these animals was done in compliance with the law for protection of cruelty against animals.

Blood was taken from the jugular vein into vials with 3.8 % sodium citrate to obtain a 1 : 9 ratio with blood. Samples were immediately centrifuged at 2500 RPM for 5 min, plasma samples were then immediately frozen and kept at -86°C until analysis. Coagulation parameters were measured on a Start 4 coagulometer, STAGO diagnostics. Prothrombin time (Quick test), APTT, thrombin time, fibrinogen content and factor VIII and IX content were assessed. Every sample was measured as a duplicate. Prothrombin was measured using calcium thromboplastin to start the clotting reaction (Neoplastine CI Plus). APTT assessment was based on plasma re-calcification in the presence of an activator and partial thromboplastin (Cephascreen). Thrombin time measurement was performed with a predetermined quantity of thrombin (STA-Thrombin). Fibrinogen was determined in diluted plasma in the presence of excessive thrombin (Fibri-Prest Automate) where clotting time then correlates with the level of plasma fibrinogen. Factor VIII and IX concentrations were detected using the method for APTT measurement and factor deficient plasma. All reagents used were obtained from A.L. Instruments (Cesky Tesin, Czech Republic).

Statistical analysis was performed with Unistat 5.6 for Excel. One way ANOVA and Tukey's HSD test were applied to analyse data.

Table 1. Coagulation times in fallow deer

	PT (s)	APTT (s)	TT (s)
Mean – all	20.99	33.76	24.78
SD	2.7	6.0	3.2
Mean – females	20.16	31.42	23.33
SD	2.5	4.5	4.2
Mean – males	21.71	37.66	25.64
SD	2.6	6.4	2.6

PT = prothrombin time, APTT = activated partial thromboplastin time, TT = thrombin time

RESULTS

Mean values for clotting parameters are given in Table 1 (coagulation times) and Table 2 (fibrinogen content and concentration of factors). Data were compared for the different gender and age of examined animals. The first statistically significant difference was found between genders in APTT values; males had significantly longer clotting times compared to females ($P < 0.05$). The second statistically significant difference was found in fibrinogen levels, where a concentration of 7.7 g/l in one animal from the six months of age group differed from all the animals from other age categories ($P < 0.05$) (data not shown).

DISCUSSION

There is sufficient information on haematological and biochemical parameters in wild ruminants (Peinado et al., 1999; Vengust et al., 2002). However, data on haemostasis in these species is rather scarce. Only Sutherland et al. (1985) have

Table 2. Fibrinogen content and factor VIII and IX concentrations in fallow deer

	Fibrinogen (g/l)	Factor VIII (%)	Factor IX (%)
Mean – all	1.94	198.42	169.91
SD	1.3	6.4	30.5
Mean – females	2.02	199.71	166.77
SD	1.7	1.0	35.0
Mean – males	1.92	196.83	173.73
SD	0.4	9.5	25.4

reported data for PT, APTT and fibrinogen content in fallow deer. They also assessed activated clotting time and fibrinogen degradation products, measurements of which were not included in our research. Unfortunately, their coagulation parameters ranges did not include a large enough representative group of animals.

Comparing the data, the fibrinogen content obtained in this study (1.94 g/l) corresponds with the results reported by Sutherland et al. (1985) (1.51 g/l). An interesting observation was made in young animals (six months old), where 7.7 g/l of fibrinogen was detected (repetitive measurement). As we had only one animal of this age, it is not clear whether this result is only an exception or this very high concentration of fibrinogen is a typical feature of young fallow deer. In humans, it is reported that children can have a different structure and composition of fibrinogen leading to its decreased polymerisation (Miller et al., 2004; Monagle et al., 2010), but the level is usually comparable to that typical for adults (1.5–3.5 g/l). This result thus has to undergo further investigation and extended measurement on a more numerous sample of young animals to confirm or reject it.

Our APTT results are also in agreement with Sutherland et al. (1985). On the other hand, the data on PT differ to a certain extent. Values obtained in our study show a longer clotting time (20.99 s) compared to the 12.9 s reported by Sutherland et al. (1985). We attribute this to the possible stress associated with handling the animals and keeping them yarded before the transportation. However, the times obtained are not sufficiently high to indicate haemorrhages or coagulation difficulties in the examined animals, also with regard to the probably physiological levels of the rest of haemostatic parameters assessed.

Surprisingly, a significant difference was found in APTT between males and females. It may be hypothesized that higher clotting times in males compared to females represent a physiological disadvantage of this gender to deal with injuries (e.g., due to frequent fighting in the heat period). On the other hand, females are supposed to be less amenable to delivery associated bleeding.

Reference values for other haemostatic parameters measured in this study (i.e., TT, factor VIII and factor IX) had not yet been reported for fallow deer. Thrombin time (24.78 s) resembles values obtained for domestic ruminants (15.3–27.0 s) (Lewis, 1996). Values of concentrations of factors VIII and

IX obtained in this experiment are consistent with the general experience in many mammals which have concentrations of these factors higher than humans (Lewis, 1996).

There are not many species of hoofed game for which there is sufficient information on blood clotting processes. Based on the available data, the species comparable to fallow deer include red deer (*Cervus elaphus*), mouflons (*Ovis musimon*) or domestic ruminants (Sutherland et al., 1985; Lewis, 1996; Doubek et al., 2003). Interestingly, the obtained values vary significantly even in these close relatives, e.g., PT is 14–18 s in mouflon, while it is stated to be 45–55 s in red deer (Doubek et al., 2003). Values reported by different authors vary considerably making it very hard to establish valid physiological reference ranges. For example, Doubek et al. (2003) claim TT to be approximately 6–11 s in ruminants, while Lewis (1996) gives values of approximately 15.3–27.0 s. Similarly, Doubek et al. (2003) report PT in red deer to be 45–55 s, while Sutherland et al. (1985) found PT to be 16.3 s.

Parameters of haemostasis are important for comparative physiology and for the monitoring of physiological or pathological processes. The reference values obtained in this study can be useful in the clinical and laboratory examination of fallow deer and may help in distinguishing causes of haemorrhage, including anticoagulant intoxications and infectious diseases leading to sepsis resulting in disseminated intravascular coagulation.

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Received: 2010–09–24

Accepted after corrections: 2011–03–06

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