Blood coagulation times in the European brown hare (Lepus europaeus)

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Background: Many causes of mortality in the European brown hare, such as bacterial and viral infections, anticoagulant poisoning, and trauma, may result in hemorrhage. There are, however, no reference values concerning blood clotting in this species. Objectives: The aim of this study was to determine reference values for blood coagulation times and related parameters in healthy European brown hares. Methods: Blood samples from 30 clinically healthy adult hares (15 males and 15 females) were obtained. Hares were physically restrained for blood collection from the cephalic vein into tubes containing citrate and EDTA. Results: Mean ± SD were obtained for thrombin time (TT) (13.97 ± 1.37 seconds), prothrombin time (PT) (13.32 ± 2.15 seconds), activated partial thromboplastin time (APTT) (16.73 ± 1.86 seconds), fibrinogen concentration (2.98 ± 1.06 g/L), and platelet count (355.28 ± 128.73 × 10⁹/L). Conclusions: Reference values for blood coagulation times and other parameters associated with blood clotting will be useful in the laboratory evaluation of hemorrhage in the European brown hare. (Vet Clin Pathol. 2007;36:361–363)

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Key Words: Clotting time, coagulation, fibrinogen, hemostasis, platelet, reference interval

The European brown hare (Lepus europaeus) is one of the most important game animal species of Central Europe, with bag records amounting to 1.1 million in the Czech Republic in 1971. Bag records stood only at 84,673 in 2001. This shows a major population decline during the past 3 decades, the causes of which are not fully understood and are generally assumed to be multifactorial. In an analysis of mortality of 2269 wild European brown hares in the Czech Republic, it was found that dietary, parasitic, infectious, toxic, and traumatic causes were responsible for 25%, 25%, 30%, 10%, and 10% of deaths, respectively. Bacterial diseases such as pasteurellosis, pseudotuberculosis, brucellosis, tularemia, and staphylococcosis were diagnosed most frequently. Bacterial infections were responsible for mortality in 15% of hares in Switzerland. A calcivirus causing European brown hare syndrome also has resulted in high mortality and is associated with dramatic declines in populations across Europe.

In the European brown hare clinical manifestation of infections, such as pasteurellosis, tularemia, and European brown hare syndrome, may include hemorrhagic sepsis. Other common causes of hemorrhage in wildlife include trauma and anticoagulant poisoning. Thus, clinical examination of live animals and laboratory screening tests for coagulation disorders may prove useful in distinguishing among causes of hemorrhage in the European brown hare. However, we have found no published reference values concerning blood clotting in this species. The topic of anticoagulation in the European brown hare is novel and is likely to become relevant in the near future. Because many potential causes of mortality have a hemorrhagic component, it was the objective of this study to determine reference values for blood coagulation times and related parameters in the European brown hare.

Blood samples from 30 European brown hares (15 males and 15 females) were obtained in January 2006. The animals were trapped live in hunting grounds around Prerov (South Moravia, Czech Republic). The animals were kept on the premises of the Interlov Company (Prerov) and later used for repopulation purposes in Italy. Sampling of these animals was performed in compliance with laws for the protection of animals against cruelty and was approved by the Ethical Committee of the University of Veterinary and Pharmaceutical Sciences Brno, Czech Republic. At the time of blood collection the hares appeared healthy, were in excellent nutritional state, and were certified free of tularemia and brucellosis based on agglutination tests. All animals were adults, according to the Stroh sign, ie, clinical assessment of distal ulnar epiphysyeal closure at 7–8 months of age.

Hares were physically restrained for blood collection from the cephalic vein. First, 0.5 mL of blood was drawn into a 1.5 mL tube containing 3.8% sodium citrate solution in a 1:9 ratio with blood. Second, 0.5 mL of blood was collected into a 1 mL tube with EDTA (Meus, Piove di Sacco, Italy) for platelet counts. The citrated sample was centrifuged and plasma removed within 15 minutes. The plasma was frozen and stored at −80°C and analyzed within a week. The EDTA sample was cooled to 4°C and analyzed on the day of collection. Plasma and EDTA samples were trans-
Coagulation Times in European Brown Hares

Table 1. Blood coagulation results for 30 healthy adult European brown hares (Lupus europaues).

<table>
<thead>
<tr>
<th>Analyte (units)</th>
<th>Mean ± SD</th>
<th>Minimum–Maximum</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thrombin time (seconds)</td>
<td>13.97 ± 1.37</td>
<td>12.3–16.4</td>
</tr>
<tr>
<td>Prothrombin time (seconds)</td>
<td>13.32 ± 2.15</td>
<td>9.4–16.2</td>
</tr>
<tr>
<td>Activated partial thromboplastin time (seconds)</td>
<td>16.73 ± 1.86</td>
<td>13.8–19.8</td>
</tr>
<tr>
<td>Fibrinogen (g/L)</td>
<td>2.98 ± 1.06</td>
<td>1.91–4.55</td>
</tr>
<tr>
<td>Platelets (×10⁹/L)</td>
<td>355.28 ± 128.73</td>
<td>194–523</td>
</tr>
</tbody>
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ported to the laboratory using dry ice and a thermobox, respectively.

Thrombin time (TT), prothrombin time (PT), activated partial thromboplastin time (APTT), and fibrinogen concentration were analyzed using a Diaclot–C1 semiautomatic coagulometer (Dialab, Prague, Czech Republic). Reagents for TT, PT, APTT, and fibrinogen as well as control and calibration plasma were obtained from TECO GMBH (Neufahrn, Germany). Platelet count was determined using a semiautomated electronic impedance analyzer (MEK-5208K, Nihon Kohden, Japan).

Statistical analysis was performed with STATISTICA for Windows 7.0 (StatSoft, Tulsa, OK, USA). Data normality and homogeneity of variances were evaluated by the Kolmogorov-Smirnov and Levene’s tests, respectively. A one-way ANOVA and a nonparametric Kruskal-Wallis test were used for statistical comparisons of differences between males and females. Values of \( P < .05 \) were considered statistically significant for all tests. Data for all parameters were distributed normally. Mean ± SD and minimum-maximum values for coagulation parameters and platelet counts are shown in Table 1. Statistically significant differences were not found between females and males; therefore, these values were combined.

To our knowledge, no one has previously evaluated and published blood coagulation times and other parameters associated with blood clotting in the European brown hare. A comprehensive survey of hematology and serum chemistry values in the European brown hare was published; however, the platelet count was the only parameter associated with blood coagulation included in the study. The platelet counts obtained in this study corresponded to reference values reported in the survey.

Owing to the lack of data on normal blood coagulation in the European brown hare as well as in other species of hares, our results may only be compared with another related species—the rabbit. Reported blood coagulation values for PT and APTT in New Zealand white rabbits were 7.5 ± 1.5 seconds and 15.7–42.7 seconds, respectively.10,11 The PT is longer in the European brown hare than in the rabbit, while the opposite is true for the APTT. TT values of 22.9 ± 5.9 seconds in the rabbit are longer than those in the European brown hare.13 Rabbits are frequently the subjects of research. Experiments with rabbits have been performed to obtain direct evidence that endotoxin-induced disseminated intravascular coagulation (DIC) stems primarily from exposure of circulating blood to tissue factor.12 Skin bleeding times also may be measured to evaluate hemostasis.13 In our study, skin bleeding times were not determined in European brown hares because the owners, intending to sell them, insisted strictly on allowing no visible harm to the animals.

Experimental rabbits inoculated with a suspension of the calicivirus that causes viral hemorrhagic disease (VHD) were sampled every 6 hours postinoculation in order to assess hemostatic functions.14 Typical laboratory signs of DIC, including thrombocytopenia and prolonged PT and APTT were detected at 30 hours postinoculation and later. Surprisingly, fibrinogen concentrations remained high, possibly because fibrinogen is also an acute phase reactant in rabbits and its plasma concentration may be increased in inflammatory conditions.14 (The authors have observed a rise in fibrinogen levels following experimental infection of European brown hares by Francisella tularensis [unpublished data].) European brown hare syndrome is caused by a calicivirus related to the agent of the VHD in rabbits,4,6,15 such that similarities exist in the pathogenesis of both of these diseases of lagomorphs. Similar changes in hemostasis, including the development of DIC, may be expected in the European brown hare syndrome. The reference values obtained in this study will be valuable in assessing DIC in European brown hares with calicivirus infections as well as DIC associated with other underlying disorders, including massive trauma, severe hemolysis, effects of endotoxins, bacterial infections producing sepsis, tissue necrosis or inflammation, and heavy metal toxicosis.16,17

In summary, the reference values obtained in this study will be useful in the clinical and laboratory examination of the European brown hare and will help in distinguishing among causes of hemorrhage, including anticoagulant intoxications and infectious diseases, such as European brown hare syndrome, pasteurellosis, and tularemia, leading to sepsis resulting in DIC.

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References


