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Haematological tests in sheep health management☆

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ABSTRACT

The evaluation of sheep complete blood cell count (CBC) can be of significant aid to clinical examination. Accurate assessment of haematologic data depends on the proper collection, preparation and transportation of blood samples. Blood should be drawn from animals at rest, calm and well restrained at the time of sampling, in order to avoid artifactual changes, such as a stress leucogram caused by endogenous steroid or epinephrine release or haemolysis from traumatic collection. Proper venipuncture is important to avoid contamination by tissue thromboplastin, which encourages clot formation and invalidates haemostatic function tests. Tubes must be filled to capacity to ensure the proper blood–anticoagulant ratio. When haemostasis tests are indicated, blood should be carefully transferred to vials containing anticoagulant, in order to prevent haemolysis, which precludes the use of evacuated tubes. Blood samples for haematologic studies are best processed as soon as possible after collection, but if a delay is expected they should be refrigerated at 4 °C and for a maximum period of 24 h (with the exception of platelet counts which should be performed within 4–6 h after sample collection), while air-dried blood smears must be prepared immediately after sampling to be stained with Wright or any modified Romanovsky stain. Complete blood counts can be performed either manually or with the aid of sophisticated instrumentation. Automated haematology instruments provide rapid cell counts of blood, haemoglobin calculation, potentially useful erythrocyte indices, but they cannot detect morphologic abnormalities and haemoparasites. Red blood cell counting and morphological changes are useful when evaluating anaemia. The differential white blood cell count is more important than the total white blood cell count, because increases and decreases in individual cell types may occur simultaneously, leaving total count unchanged. Overall, CBC can provide valuable information to the sheep clinician. Moreover, evaluation of a well-prepared blood film is an essential adjunct to interpretation of all cell counts, allowing detection of significant morphologic abnormalities or presence of haemoparasites.

1. Introduction

Haematological diagnostic techniques have become an essential part of the minimum data base for diagnostic investigation of medical problems in sheep. The ever expanding use of sophisticated equipment, that can be used for processing small ruminant blood samples, and the increasing owner awareness regarding the benefits of clinicopathological diagnostic workup, have established the complete blood cell count (CBC) as an important part of sheep case management (Roger, 2008). The interpretation of the haematological profile in combination with the findings of clinical examination and the results of other diagnostic tests may point to a specific differential diagnosis or suggest a prognosis.

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The sheep haematological profile includes the determination of haematocrit (Hct) or packed cell volume (PCV), of haemoglobin, of red blood cell indices, of total cell counts, of differential cell counts and the evaluation of erythrocyte, of leucocyte and of platelet morphology by microscopic examination of stained blood films (Kramer, 2000; Stockham and Scott, 2002a; Brockus and Andreasen, 2003).

2. Collection, handling and storage of blood samples from sheep

The diagnostic value of a blood sample is its ability to reflect the effects of disease on the blood cells and platelets. Blood composition is not static, but rapid changes may occur as a response to various physiological events triggered by stress, such as splenic contraction and demargination of neutrophils. These processes that are usually induced by animal handling and restraining, often produce physiological changes that may be erroneously interpreted. For example, stress-induced steroid or epinephrine release could produce transient neutrophilia that may be mistaken for an inflammatory leucogram and increased PCV due to splenic contraction that may be mistaken for dehydration (Stockham and Scott, 2002b; Latimer and Prasse, 2003; Jones and Allison, 2007).

Physical damage to blood constituents during collection is the most common cause of poor quality blood samples. The fragility of erythrocytes and the aggregability of platelets are quite unpredictable and vary among individual animals and pathological processes. Depending on the size of the animal, an 18 or 20 gauge and 1.5–2 in. needle should be used; the blood should be collected into evacuated tubes or aspirated gently using a syringe. The jugular veins are the preferred sites of venipuncture, because of their size and accessibility. Use of a consistent and accurate technique, such as rapid needle placement and quick sample collection, minimizes artifactual changes like iatrogenic haemolysis, tissue thromboplastin contamination and platelet activation (Kramer, 2000; Stockham and Scott, 2002a; Morris, 2008a).

The selection of a particular anticoagulant depends on the type of diagnostic test(s) to be performed. Commonly used anticoagulants include ethylenediaminetetra-acetic acid (EDTA), heparin and sodium citrate. Regardless of the sample volume, it is imperative to maintain the appropriate ratio of blood to anticoagulant. Excessive EDTA concentration may cause erythrocyte shrinking, thus invalidating various parameters, such as the PCV, the mean corpuscular volume (MCV) and the mean corpuscular haemoglobin concentration (MCHC). The preferred anticoagulant for the CBC is EDTA, because it preserves cell morphology. Sodium citrate is used for any test involving clotting times and factors (Morris, 2008a,b). Heparinised samples are of limited use, because white blood cell (WBC) staining is poor, however, basic red blood cell (RBC) counts and indices can be assessed.

After sample collection, tubes should be inverted or rolled gently several times to ensure adequate anticoagulant mixing and the samples processed as soon as possible (Brockus and Andreasen, 2003; Morris, 2008a,b). Optimally, assays should be carried out immediately; however, if this is not possible, the samples must be refrigerated at 4 °C, because, at room temperature, RBC swelling occurring after 6–24 h, may lead to aberrations, such as increased PCV and decreased MCHC. Properly refrigerated samples can be assayed up to 24 h after collection, with the exception of platelet counts, which must be performed within 6 h after sample collection (Jones and Allison, 2007; Morris, 2008a). Blood samples forwarded to post to a laboratory may produce artefactual results, therefore it is imperative to ensure packing with ice or cold packs in insulated containers to minimize these effects (Topper and Welles, 2003; Jones and Allison, 2007).

It is important to prepare several air-dried blood films immediately after sample collection and send them to the diagnostic laboratory along with the refrigerated aliquots. Despite the fact that the quality of these smears may vary, they can be very useful to distinguish true cell morphology changes that are important, from alterations caused by sample ageing. In addition, the platelet counts can be crudely assessed using the air-dried blood films, by comparing their number per oil immersion field with the number of red or white blood cells (Kramer, 2000; Stockham and Scott, 2002a; Morris, 2008a).

In modern veterinary practice, use of automated haematology analysers is increasing and these instruments are becoming available for several farm animal species, including sheep. However, it is important to use instruments calibrated for use in sheep, because variations in the size of RBCs among animal species can lead to erroneous results. Despite the rapid and accurate determination of cell counts and RBC indices, which cannot be estimated manually, these are not able to detect morphological abnormalities or haemoparasites. Thus, the importance of the simultaneous examination of blood films is emphasized (Brockus and Andreasen, 2003; Jones and Allison, 2007).

3. Erythrocyte parameters and morphology

The parameters included in RBC evaluation are the PCV or haematocrit (HCT), RBC count, haemoglobin, MCV and MCHC. As mentioned previously, it is important to examine also a stained blood film, in order to detect morphology changes and parasites. The RBCs of small ruminants are relatively small compared to those of other species, thus they are often erroneously interpreted when using haematology instruments calibrated for use in other species. This is particularly true for impedance analysers, where RBCs are often counted as platelets and falsely decreasing PCV values (Kramer, 2000; Morris, 2008a). Similar errors can also occur with *in vitro* haemolysis, another problem encountered often when handling ovine blood samples. The microhaematocrit is the easiest way to calculate PCV and to assess RBC count manually and should always be performed, even if automated instruments are available. A significant difference between the PCV and microhaematocrit values is an indication of inaccurate determination by the analyser. The RBC indices, MCV, MCH and MCHC, provide information about the average cell size, haemoglobin content and haemoglobin concentration, respectively. An increased MCV (macrocytosis) is an indication of regenera-