Diagnostic approach of anemia in ruminants

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ABSTRACT. Anemia in ruminants is an abnormal condition characterized by the decrease of the hematocrit (Packed Cell Volume, PCV), the mass of erythrocytes (Red Blood Cells, RBCs) and/or hemoglobin. Anemia is classified as hemolytic, hemorrhagic or anemia caused by the decreased production of erythrocytes; the first two categories are characterized by a regenerative response. Hemorrhagic anemia can be caused by ectoparasites or parasites of the gastrointestinal system, hemorrhagic bowel syndrome, abomasal ulcers, vena cava thrombosis as well as from the genitourinary tract. In addition, primary and secondary hemostatic disorders can be accompanied by hemorrhagic anemia. Hemoparasites, toxins produced from Clostridium perfringens type D and Clostridium hemolyticum and leptospirosis are some of the causes of hemolytic anemia. Furthermore, certain plants, drugs or heavy metals and lack of certain trace elements can cause hemolysis. Immune-mediated hemolytic anemia has also been reported in ruminants. The reduced production of erythrocytes can be caused by deficiency of vitamin B12 or iron, as well as by chronic diseases. Pathologic conditions of bone marrow like inflammatory or neoplastic cells filtration and hypoplasia or aplasia of bone marrow are related to reduced production of erythrocytes. After laboratory confirmation by complete blood count analysis, history taking, clinical examination
of the animal and specific test depending on the case, are required for the diagnostic approach of anemia and especially for etiological diagnosis. Tachycardia, tachypnea, icterus, mucosal pallor as well as specific symptoms of the underlying disease are observed during the clinical examination of the animal. FAMACHA technique is widely used for the clinical diagnosis and the assessment of the severity of anemia. With respect to complete blood count, apart from the hematocrit, hemoglobin concentration, erythrocytes indices as Mean Corpuscular Volume (MCV) and Mean Corpuscular Hemoglobin Concentration (MCHC) are contributing to the classification of anemia. The size and the shape of the erythrocytes, the appearance of inclusions and reticulocytes, which are indicative of regenerative anemia, are evaluated in blood smear. Rarely, examination of bone marrow is carried out, for the differentiation of anemia as regenerative or non-regenerative. In particular, the confirmation of immune- mediate anemia is based on Coomb’s test for the detection of autoagglutination. Except from hemorrhage, blood loss is detected at urinalysis or feces microscopy for the presence of blood and/or parasites. Finally, serological and molecular techniques for the detection of infectious agents, as well as specific toxicological analysis are performed in various biological materials.

**Keywords:** anemia, ruminants, diagnostic approach

**ΠΕΡΙΛΗΨΗ.** Η αναιμία στα μηρυκαστικά είναι παθολογική κατάσταση, η οποία χαρακτηρίζεται από τη μείωση του αιματοκρίτη (Packed Cell Volume, PCV), του αριθμού των ερυθροκυττάρων (Red Blood Cells, RBCs) ή και της αιμοσφαιρίνης. Η αναιμία χαρακτηρίζεται ως αιμολυτική, αιμορραγική ή αναιμία που οφείλεται στη μειωμένη παραγωγή των ερυθροκυττάρων, με τις δύο πρώτες κατηγορίες να χαρακτηρίζονται από αναγεννητικότητα. Η αναιμία λόγω απώλειας αίματος μπορεί να οφείλεται σε εξωεπαρκείς ή ενδοεπαρκείς του γαστρεντερικού συστήματος στο σύνδρομο του αιμορραγικού εντέρου, σε έλκη του ηνύστρου, σε έμφραξη της οπίσθιας κοίλης φλέβας καθώς και σε αιμορραγία από το υποφυσικό σύστημα. Ακόμη, πρωτογενείς και δευτερογενείς διαταραχές της αιμόστασης μπορεί να συνοδεύονται από αιμορραγική αναιμία. Στα αίτια της αιμολυτικής αναιμίας περιλαμβάνονται τα αιμοπαράσιτα, οι τοξίνες που παράγεται από τα *Clostridium perfringens* τύπου D και *Clostridium hemolyticum* και η εξωπαρασίτηση. Η αιμολυτική αναιμία μπορεί να αναφέρεται στα μηρυκαστικά. Η μειωμένη παραγωγή ερυθροκυττάρων οφείλεται σε έλλειψη της βιταμίνης Β12 ή του σιδήρου, καθώς και σε κρούσματα. Παθολογικές καταστάσεις του μυελού των οστών όπως η διήθηση από φλεγμονικά και νεοπλασματικά κύτταρα ή/και η υποκλίση της αιμόστασης και η ελλείψη ιχνοστοιχείων. Η μειωμένη παραγωγή ερυθροκυττάρων μπορεί να οφείλεται σε εκτός-κυττάρως αναγεννητικότητα. Για τη διαγνωστική προσέγγιση της αναιμίας μετά την εργαστηριακή επιβεβαίωση με τη γενική εξέταση του αίματος και κυρίως για την αιτιολογική διάγνωση απαιτείται η λήψη ιστορικού, η κλινική εξέταση του ζώου και ειδικές κατά περίπτωση εξετάσεις. Κατά την κλινική εξέταση του ζώου παρατηρούνται ταχυκαρδία, ταχύπνοια, ίκτερος, ωχροί βλεννογόνοι αλλά και τα ειδικότερα συμπτώματα του υποκείμενου νοσήματος. Η τεχνική FAMACHA έχει ευρέως χρησιμοποιηθεί για την κλινική διάγνωση και τον προσδιορισμό της βαρύτητας της αναιμίας. Επιπλέον, ορισμένα φυτά, φάρμακα ή βαρέα μέταλλα μπορούν να προκαλέσουν αιμόλυση των ερυθροκυττάρων καθώς και η έλλειψη ιχνοστοιχείων. Η αναιμία μπορεί να συνοδεύεται με αιμορραγία, η οποία μπορεί να αναφέρεται στην εξωεπαρκή της συστήματος και την ενδοεπαρκή της συστήματος. Η μειωμένη παραγωγή ερυθροκυττάρων μπορεί να οφείλεται σε εξωεπαρκές ή ενδοεπαρκές του γαστρεντερικού συστήματος. Τέλος στα διάφορα βιολογικά υλικά πραγματοποιούνται ορολογικές και μοριακές τεχνικές για την ανίχνευση παθογόνων παραγόντων καθώς και ειδικές εξετάσεις για την ανίχνευση τοξινών.

**Λέξεις ευρετηρίασης:** αναιμία, μηρυκαστικά, διαγνωστική προσέγγιση.
INTRODUCTION

Anemia, by definition, corresponds to the reduction of blood oxygen and it is characterized by a decrease in hematocrit, erythrocyte mass and/or hemoglobin concentration leading to tissue hypoxia. In practice anemia is defined as the concentration of hemoglobin below the lower reference limit.

Anemia can be classified either based on the ensuing pathogenetic mechanisms thus characterized as hemorrhagic, hemolytic and anemia caused by the decreased production of erythrocytes or based on the regenerative response of bone marrow and consequently characterized as regenerative or non-regenerative anemia.

The presence of hypoxia in renal erythropoietin producing cells, as a consequence of anemia, dynamically alters the concentration of circulating erythropoietin. Erythropoietin is an early identified humoral factor, an erythropoietic glycoprotein hormone that induces proliferation and differentiation of erythroid progenitors, leading to red blood cell production (Souma et al., 2015).

At first, anemia is always non-regenerative because it takes some days for mature erythrocytes to be released to the peripheral blood. Therefore, evidence of regeneration should become apparent within 4-5 days.

Regeneration is mainly observed in acute blood loss or in hemolytic anemia while it is rather unlikely in cases where anemia is related to a chronic disease or by impaired erythrocyte production by the bone marrow (Ogilvie, 1998).

ETIOLOGY

Hemorrhagic anemia

Anemia due to blood loss can be caused by intense ectoparasitism (Stromberagn and Gyillot, 1987; Shemanchuk et al., 1960). Similarly, parasitism of the gastrointestinal tract is a common cause of blood loss anemia in ruminants as in other species. Different parasitic phyla have been identified as causative agents of anemia such as nematodes in small ruminants (Baldissera et al., 2015; Bordoloi et al., 2012; Vatta et al., 2002) and in cattle (Favero et al., 2016; Van Aken et al., 1997) with Haemonchus contortus referred as the most common species causing blood loss anemia as well as trematodes including Fasciola hepatica in small ruminants (Saleh, 2008; Maltinez- Moreno et al., 1997; Knight, 1980) and in cattle (Lotfollahzadeh et al., 2008; Ross et al., 1966) and Paramphistomum cervi, as well (Dorny et al., 2011). In severe cases of winter dysentery, a disease with unconfirmed causal agent, fresh blood clots have been reported to be evidenced in the feces of affected calves (Divers and Peek, 2008). Other clinical conditions of the gastrointestinal tract associated with blood loss anemia are the hemorrhagic bowel syndrome caused by Clostridium perfringens type A (Braun et al., 2010) and abomasal ulcers (Ok et al., 2001; Vatn and Ulvund, 2000).

Vena cava thrombosis in adult dairy cattle or in feedlot cattle fed a high-carbohydrate diet is also clinically manifested by pale mucous membranes, hemoptysis, and epistaxis (Breeze et al., 1976). Blood loss is also evident from the genitourinary tract presented as hematuria in the case of enzootic hematuria caused by urinary bladder tumors secondary to chronic bracken fern toxicity (McKenzie, 1978) as well as in pyelonephritis caused by Corynebacterium renale and E. coli in cows (Braun et al., 2008). Moreover, acute blood loss, sometimes severe, can result from injuries caused by obstetrical manipulations more often in cows that have been in labor for several hours and when the birth canal is dry and non-lubricated (Radostitis et al., 2006). In addition, anemia can be caused by tumors (Stock et al., 2011). Rupture of an ovarian granulosa cell tumor followed by hemoperitoneum or spleen rupture after infiltration by lymphosarcoma (Divers and Peek, 2008), is a rather uncommon condition in cows (Masseau et al., 2004). Body cavity effusions usually result from hemo-static disorders (Radostitis et al., 2006). Internal hemorrhages caused by massive thrombocytopenia have been attributed to Bovine Virus Diarrhea- Mucosal Disease (BVD- MD) virus (Dabak et al., 2007). Secondary hemostatic disorders such as rodenticide intoxication are very rarely reported in ruminants. A case of rodenticide toxicity in lambs that accidentally gain access to baits for rodent control was reported as sudden death followed epistaxis, respiratory distress and edemas in different body parts (Del Piero and Poppenga, 2006). Experimentally dosing of carbon tetrachloride, in goats resulted in hepatic damage and decreased of blood clotting activity (Smith and Sherman, 2009).

The most frequent causes of blood loss anemia per ruminant species are presented in Table 1.
Hemolysis also results from toxins produced by *Clostridium perfringens* type D causing yellow lamb disease (Gianniti et al., 2014) and *Clostridium hemolyticum* that causes bacillary hemoglobinuria in sheep and cows (Tagaki et al., 2009; Randhawa et al., 1995).

The primary lesions of the pathogenic *Leptospira* spp circulating in the bloodstream, concern the endothelium of small blood vessels, which leads to localized ischemia in organs mainly kidney and liver. Apart from vasculitis, *Leptospira* spp hemolysins have been implicated in the pathogenesis of anemia; in calves *Leptospira* spp hemolysin has been reported as responsible for yielding holes in the erythrocyte cellular membrane (Lee et al., 2002).

Toxicity from grazing plants *Allium* spp including onion and garlic (Heidarpour et al., 2013; Aslani et al., 2005; Rae, 1999), *Brassica* spp with the most known the broccoli and the cabbage (Xu, 1992) and *Ipomoea carnea*, a flowery plant (Tartour et al., 1973), have been associated with hemolytic anemia in ruminants. Although, long acting oxytetracycline compounds have been widely used in ruminant medicine only

<table>
<thead>
<tr>
<th>Hemolytic anemia</th>
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</table>
| Anemia due to hemolysis can be caused by parasites implicated in tick born fever including piroplasms that invade red blood cells like *Babesia* spp in small (Esmaeilnejad et al., 2012; Yeruham et al., 1998) and large ruminants (Bal et al., 2016; Trueman and Blight, 1978) and *Theileria* spp in lambs (Alani and Herbert, 1988) and cattle (Omer et al., 2002; Moll et al., 1986) and rickettsiae, mainly *Anaplasma* spp that infect granulocytes up to 90% in the peak of bacteremia as it is referred in sheep (Yasini et al., 2012), goats (Gokce and Woldehiwet, 1999) and cattle (Ashuma et al., 2013; Henniger et al., 2013). Furthermore, protozoa like *Trypanosoma* (Biryomumaisho et al., 2013; Katunguka- Rwakishaya et al., 1997; Anosa et al., 1992) and certain *Mycoplasma* species (Genova et al., 2011; Suzuki et al., 2011) can also be found in plasma and/or in erythrocytes. In addition, microfilaria, the larval stage of stephanofilarial worm, the causative agent of the bovine stephanofilarial dermatitis, enters peripheral blood circulation causing anemia by increasing erythrocytic fragility (Singh et al., 2011). Hemolysis also results from toxins produced by *Clostridium perfringens* type D causing yellow lamb disease (Gianniti et al., 2014) and *Clostridium hemolyticum* that causes bacillary hemoglobinuria in sheep and cows (Tagaki et al., 2009; Randhawa et al., 1995). The primary lesions of the pathogenic *Leptospira* spp circulating in the bloodstream, concern the endothelium of small blood vessels, which leads to localized ischemia in organs mainly kidney and liver. Apart from vasculitis, *Leptospira* spp hemolysins have been implicated in the pathogenesis of anemia; in calves *Leptospira* spp hemolysin has been reported as responsible for yielding holes in the erythrocyte cellular membrane (Lee et al., 2002). Toxicity from grazing plants *Allium* spp including onion and garlic (Heidarpour et al., 2013; Aslani et al., 2005; Rae, 1999), *Brassica* spp with the most known the broccoli and the cabbage (Xu, 1992) and *Ipomoea carnea*, a flowery plant (Tartour et al., 1973), have been associated with hemolytic anemia in ruminants. Although, long acting oxytetracycline compounds have been widely used in ruminant medicine only

**Table 1. Causes of blood loss anemia in sheep, goats and cattle**

<table>
<thead>
<tr>
<th>Ectoparasites</th>
<th>Sheep</th>
<th>Goats</th>
<th>Cattle</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Linognathus</em> spp</td>
<td>A, Y</td>
<td></td>
<td></td>
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<tr>
<td><strong>Gastrointestinal tract</strong></td>
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<tr>
<td><em>Haemonchus concortus</em></td>
<td>A, Y</td>
<td></td>
<td>Y</td>
</tr>
<tr>
<td><em>Mecistocirrus</em> spp</td>
<td></td>
<td>Y</td>
<td></td>
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<tr>
<td><em>Fasciola hepatica</em></td>
<td>A, Y</td>
<td></td>
<td>A, Y</td>
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<tr>
<td><em>Paramphistomum cervi</em></td>
<td></td>
<td></td>
<td>A, Y</td>
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<tr>
<td>Abomasal ulceration</td>
<td></td>
<td></td>
<td>Y</td>
</tr>
<tr>
<td>Hemorrhagic bowel syndrome</td>
<td></td>
<td></td>
<td>A</td>
</tr>
<tr>
<td>Winter dysentery</td>
<td></td>
<td></td>
<td>Y</td>
</tr>
<tr>
<td><strong>Respiratory tract</strong></td>
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<td></td>
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<tr>
<td>Caudal vena cava thrombosis and pulmonary embolism</td>
<td></td>
<td></td>
<td>A</td>
</tr>
<tr>
<td><strong>Genitourinary tract disease</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Enzoonotic hematuria</td>
<td></td>
<td></td>
<td>A</td>
</tr>
<tr>
<td><strong>Others</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Injuries</td>
<td></td>
<td></td>
<td>A</td>
</tr>
<tr>
<td>Tumor</td>
<td></td>
<td></td>
<td>A, Y</td>
</tr>
<tr>
<td>Hemostatic disorders</td>
<td>Y</td>
<td></td>
<td>A</td>
</tr>
<tr>
<td>Carbon tetrachloride</td>
<td></td>
<td></td>
<td>A</td>
</tr>
</tbody>
</table>

*Sheep: Young (Y) < 12 months, Adult (A) >12 months; Goats: Young (Y) < 12 months, Adult (A) >12 months; Cattle: Young (Y) < 12 months, Adult (A) >12 months*
experimentally induced toxicosis has been reported. It seems that oxytetracycline may penetrate erythrocytes interacting with hemoglobin and thereby significantly reducing the erythrocyte count and hemoglobin concentration (Chi et al., 2010).

Moreover, natural occurring cases of anemia associated with toxicosis by heavy metals such as copper (Cregar et al., 2012; Bundza et al., 1982; Stogdale, 1978), zinc (Allen et al., 1983) and arsenic (Keshavarzi et al., 2015; Rana et al., 2010) have been reported in ruminants. Although, anemia has been reported in natural cases of lead poisoning in cows (Schlerka et al., 2004), it has not been observed in experimentally lead poisoned sheep were low doses of lead where administered (Polizopoulou, 1991). In addition, poisoning by nitrate resulted in methemoglobinemia with methemoglobinuria, in sheep (Hindson and Winter, 1996). On the other hand, nutritional deficiency in copper and selenium has been associated with formation of Heinz bodies indicative of erythrocyte oxidative damage in lambs. Moreover, copious exercise seems to further reveal the hematological impact of these trace element deficiencies (Draksler et al., 2002; Suttle et al., 1987). Similarly, anemia with Heinz body presence has been reported in hypophosphatemic cows (Jubb et al., 1990).

Rarely autoimmune hemolytic anemia is reported in young, as well in adult ruminants after vaccinations; a polyvalent botulism vaccine has been incriminated for such a condition in a cow (Yeruham et al., 2003). Similarly, antigens of the BVD- MD vaccine seem to elicit antibodies that bind not only to peripheral blood

Table 2. Causes of hemolytic anemia in sheep, goats and cattle

<table>
<thead>
<tr>
<th>Infections Agents</th>
<th>Sheep</th>
<th>Goats</th>
<th>Cattle</th>
</tr>
</thead>
<tbody>
<tr>
<td>Babesia spp</td>
<td>A, Y</td>
<td>A</td>
<td>A, Y</td>
</tr>
<tr>
<td>Theileria spp</td>
<td>Y</td>
<td></td>
<td>A, Y</td>
</tr>
<tr>
<td>Anaplasma spp</td>
<td>A</td>
<td>A</td>
<td>A, Y</td>
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<tr>
<td>Trypanosoma spp</td>
<td>A, Y</td>
<td>A</td>
<td>A, Y</td>
</tr>
<tr>
<td>Mycoplasma spp</td>
<td>A</td>
<td></td>
<td>A</td>
</tr>
<tr>
<td>Bovine stephanofilarial dermatitis</td>
<td></td>
<td></td>
<td>A</td>
</tr>
<tr>
<td>Yellow lamb disease</td>
<td>Y</td>
<td></td>
<td>A</td>
</tr>
<tr>
<td>Bacillary hemoglobinuria</td>
<td>A</td>
<td></td>
<td>A</td>
</tr>
<tr>
<td>Leptospirosis spp</td>
<td>Y</td>
<td></td>
<td>A</td>
</tr>
<tr>
<td>Toxic Agents</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Allium spp</td>
<td>A</td>
<td>A</td>
<td>A, Y</td>
</tr>
<tr>
<td>Brassica spp</td>
<td></td>
<td></td>
<td>A</td>
</tr>
<tr>
<td>Ipomoea carnea</td>
<td>A</td>
<td></td>
<td>Y</td>
</tr>
<tr>
<td>Long-acting oxytetracycline</td>
<td>A</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Copper</td>
<td>Y</td>
<td></td>
<td>A</td>
</tr>
<tr>
<td>Zinc</td>
<td>A</td>
<td></td>
<td>A</td>
</tr>
<tr>
<td>Arsenic</td>
<td>A</td>
<td></td>
<td>A</td>
</tr>
<tr>
<td>Lead</td>
<td></td>
<td></td>
<td>A</td>
</tr>
<tr>
<td>Nutrition deficiency</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Copper</td>
<td>Y</td>
<td></td>
<td>Y</td>
</tr>
<tr>
<td>Selenium</td>
<td></td>
<td>Y</td>
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<tr>
<td>Phosphorus</td>
<td>Y</td>
<td></td>
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<tr>
<td>Immune Mediated Conditions</td>
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</tr>
<tr>
<td>Vaccination</td>
<td>A, Y</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bovine colostrum fed to sheep</td>
<td>Y</td>
<td>Y</td>
<td></td>
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<tr>
<td>IMHA</td>
<td></td>
<td></td>
<td>A, Y</td>
</tr>
</tbody>
</table>

*Sheep: Young (Y) < 12 months, Adult (A) >12 months; Goats: Young (Y) < 12 months, Adult (A) >12 months; Cattle: Young (Y) < 12 months, Adult (A) >12 months
cells but also to the stem cells in the bone marrow of neonates (Deutskens et al., 2011). These antigens cause opsonization of the affected cells which enhance phagocytosis by bovine macrophages. After vaccination with Lumpy Skin Disease Virus in cows, mild regenerative hypochromic macrocytic anemia has recently been reported, probably because of slight hemolysis (Katsoulos et al., 2017). Immune-mediated hemolytic anemia has been reported in juvenile lambs fed with cow colostrum (Winter and Clarkson, 1992) while idiopathic immune medi ate anemia has been diagnosed in cases where hemolysis could not be attributed to any of the above-mentioned agents (Nassiri et al., 2011).

The causes of hemolytic anemia in ruminants are presented in Table 2.

## Anemia due decreased production of RBCs
Decrease of RBCs production is metabolically relative to essential nutrients such as cobalt which is converted by ruminal microflora to vitamin B12. Vitamin B12 acts as an essential cofactor of several enzyme systems promoting red blood cell synthesis. Thus, a vitamin B12 deficiency results to anemia. In case of sheep, it has been reported (Ulvund, 1990) that white liver disease attributed either to B12 deficiency or to hepatotoxic disease in deficient lambs was associated with a decrease in hemoglobin concentration. Similar findings have been reported in cobalt deficient goats (Al- Habsy et al., 2007). Iron deficiency is usually observed in young animals, because of their rapid growth and the large amount of iron needed for the production of hemoglobin, myoglobin and other iron-containing compounds (Green et al., 1997) as well as in ewes after parturition (Cihan et al., 2016). The most frequent cause of iron deficiency is endo- and ectoparasitism (Singh et al., 2014).

However, iron is also implicated in the pathogenesis of anemia of the chronic disease. Although, iron stores are adequate for erythropoiesis, they are sequestrated making iron unavailable for hematopoiesis leading to normocytic, normochromic non regenerative anemia. (Radostits et al., 2006). Anemia of chronic disease has been reported in cattle affected by lumpy skin disease due to the chronic inflammatory response to Capripox-virus infection, that disturbs iron metabolism. Therefore, anemia is usually mild and slowly progressive and the main hematological abnormalities concern the indexes Mean Corpuscular Volume (MCV), Mean Corpuscular Hemoglobin (MCH) and Mean Corpuscular Hemoglobin Concentration (MCHC) (Abutarbush, 2015). Similarly, ovine paratuberculosis is associated with macrocytic normochromic anemia due to the chronic inflammation (Hemalatha et al., 2013), while in goats, anemia is non-regenerative (Smith and Sherman, 2009). Although, anemia is not a common finding in Bluetongue infected sheep, few cases have been reported in sheep attributed either in vasculitis caused by the virus or chronic disease (Vasileiou et al., 2016). Apart from inflammation, neoplasia has been reported to cause anemia of chronic disease. Bracken

### Table 3. Causes of anemia of decreased erythrocyte production in ruminants

<table>
<thead>
<tr>
<th></th>
<th>Sheep</th>
<th>Goats</th>
<th>Cattle</th>
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<tbody>
<tr>
<td><strong>Nutrition deficiency anemia</strong></td>
<td></td>
<td></td>
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<tr>
<td>Cobalt/ folate</td>
<td>Y</td>
<td>A</td>
<td></td>
</tr>
<tr>
<td>Iron</td>
<td>A, Y</td>
<td>A, Y</td>
<td></td>
</tr>
<tr>
<td><strong>Anemia of chronic disease</strong></td>
<td></td>
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<tr>
<td>Lumpy skin disease</td>
<td>A</td>
<td>A</td>
<td>A</td>
</tr>
<tr>
<td>Mycoplasma paratuberculosis</td>
<td>A</td>
<td>A</td>
<td>A</td>
</tr>
<tr>
<td>Chronic toxicity</td>
<td>A</td>
<td>A</td>
<td>A, Y</td>
</tr>
<tr>
<td>Bluetongue</td>
<td>A</td>
<td>A</td>
<td>A</td>
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<tr>
<td><strong>Anemia secondary to bone marrow dysfunction or dysplasia</strong></td>
<td></td>
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</tr>
<tr>
<td>Neoplasia</td>
<td>A</td>
<td>A</td>
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</tr>
<tr>
<td>Bovine neonatal pancytopenia</td>
<td>A</td>
<td>A</td>
<td>Y</td>
</tr>
</tbody>
</table>

* Sheep: Young (Y) < 12 months, Adult (A) >12 months; Goats: Young (Y) < 12 months, Adult (A) >12 months; Cattle: Young (Y) < 12 months, Adult (A) >12 months
Picion of anemia, clinical manifestations of anemia as well as other laboratory findings are essential in order to reach etiological diagnosis. Furthermore, classification of anemia offers clinicians a diagnostic algorithm to compile a list of differentials with the ultimate goal to reach a definite diagnosis of the underlying cause (Figure 1).

Clinical manifestations and severity
Clinical signs derive from inadequate oxygen supply when compensatory mechanisms fail or even because of these mechanisms. Among them, mucous membrane pallor, exercise intolerance, tachypnea and/or dyspnea are the most commonly signs observed in anemic ruminants. Heart auscultation reveals an increased heart rate as well as functional murmurs due to blood cell turbulence disturbances (Radostits et al., 2006).

Conditions associated with anemia of reduced erythrocyte production are presented in Table 3, while the most common condition associated with anemia in ruminants in Greece are presented in Table 4.

**DIAGNOSTIC APPROACH**
Anemia is mostly a diagnosis confirmed by laboratory testing. However, diagnostic approach should include history and environmental assessment. Demographic data, such as age and breed, the prevalence of the causative agents in each geographic area along with nutrition and breeding conditions including vaccinations and antiparasitic control should be taken into consideration (Deutschens et al., 2011; Dabak et al., 2007; Aslani et al., 2005). Apart from the clinical suspicion of anemia, clinical manifestations of anemia as well as other laboratory findings are essential in order to reach etiological diagnosis. Furthermore, classification of anemia offers clinicians a diagnostic algorithm to compile a list of differentials with the ultimate goal to reach a definite diagnosis of the underlying cause (Figure 1).

**Table 4. Infectious agents that have been associated with anemia * in ruminants in Greece**

<table>
<thead>
<tr>
<th>Infectious agents</th>
<th>Species</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Haemonchus contortus</em></td>
<td>sheep, goats</td>
<td>Kouam et al., 2014</td>
</tr>
<tr>
<td><em>Besnoitia besnoiti</em></td>
<td>cattle</td>
<td>Papadopoulos et al., 2014</td>
</tr>
<tr>
<td><em>Fasciola hepatica</em></td>
<td>sheep, goats</td>
<td>Kantzoura et al., 2011; Katsoulos et al., 2011</td>
</tr>
<tr>
<td><em>Anaplasma spp</em></td>
<td>sheep, cattle</td>
<td>Giadinis et al., 2011; Giadinis et al., 2015</td>
</tr>
<tr>
<td><em>Babesia spp</em></td>
<td>sheep, goats, cattle</td>
<td>Papadopoulos et al., 1996</td>
</tr>
<tr>
<td><em>Theileria spp</em></td>
<td>sheep, cattle</td>
<td>Papadopoulos et al., 1996</td>
</tr>
<tr>
<td><em>Mycobacterium paratuberculosis</em></td>
<td>goats</td>
<td>Angelidou et al., 2014</td>
</tr>
<tr>
<td><em>Leptospira spp</em></td>
<td>sheep</td>
<td>Burriel et al., 2003</td>
</tr>
<tr>
<td><em>Yellow lamb disease</em></td>
<td>sheep</td>
<td>Gkiourtzidis et al., 2001</td>
</tr>
<tr>
<td><em>BVDV</em></td>
<td>cattle</td>
<td>Billinis et al., 2005</td>
</tr>
<tr>
<td><em>Bluetongue</em></td>
<td>sheep</td>
<td>Vasileiou et al., 2016</td>
</tr>
<tr>
<td><em>Lumpy skin disease</em></td>
<td>cattle</td>
<td>Tasioudi et al., 2016; Katsoulos et al., 2017</td>
</tr>
</tbody>
</table>

* Although the documentation of a cause-effect relationship between infection agent and anemia was not the main objective of all these publications, anemia has been observed in the affected ruminants.

Fern toxicity mostly in cattle but also in sheep results to the development of tumors and consequently to this type of anemia. Bone marrow examination revealed aplasia of the hematopoietic tissues, in these animals (Prakash et al., 1996). Neoplastic tissue seems to infiltrate bone marrow as in a case of hemophagocytic histiocytic sarcoma in a cow where neoplastic histiocytes primarily proliferated in the spleen and bone marrow (Matsuda et al., 2010). A recently observed syndrome in calves is the bovine neonatal pancytopenia in which bone marrow is aplastic and hemopoietic tissues were replaced by fat cells (Fukunaka et al., 2010).

Conditions associated with anemia of reduced erythrocyte production are presented in Table 3, while the most common condition associated with anemia in ruminants in Greece are presented in Table 4.
grazing, reduced exercise tolerance and subcutaneous edema (Gianniti et al., 2014).

To the contrary severe acute hemorrhage or blood destruction is usually presented with a more severe clinical picture, even sudden deaths, depending on the cause of anemia.

Hemorrhage is evidenced during physical examination as epistaxis, hematuria and melena (Ok et al., 2001; Xu, 1992) or denoted by the presence of ectoparasites. Hemolysis is manifested by icterus, splenomegaly and abnormal urine color (Giannitti et al., 2014; Ashuma et al., 2013; Braun et al., 2008) due to hemoglobinuria or bilirubinuria. Moreover, in case of hemostatic disorders, petechiae and ecchymoses or body cavity effusions are observed (Dabak et al., 2007).

Scoring systems of the severity of clinical patent anemia as manifested by mucous membrane pallor have been developed in ruminants. The most widely used system is called FAMACHA (FAO, 1998), an acronym of the name of the originator of the system. This system had been initially developed to identify small ruminants with anemia caused by haemonchosis, in order to selectively administer antiparasitic

**Figure 1.** Algorithm for investigation of anemia in ruminants.
increased in peripheral blood therefore serial monitoring of blood parameters is required. In case of low number of reticulocytes, monitoring is essential to find out if the anemia is non-regenerative or if there is an acute regenerative anemia. Repeating of CBC analysis is important as well as in the case of high number of reticulocytes, in order to see if the anemia does no anymore exists or if the anemia is changing from regenerative to non-regenerative. Reticulocytes in sheep and goats with basophilic stippling are easily detected in EDTA anticoagulated blood stained by Giemsa. Regeneration in ruminants is also demonstrated by macrocytosis and polychromasia while anisocytosis is quite common in healthy ruminants (Weiss and Wardrop, 2010). In response to severe anemia red blood cells contain nuclear fragments, the Howell-Jolly bodies (Alani and Herbert, 1988). Macrocytosis, is determined by calculating the Mean Corpuscular Volume (MCV) (Alani and Herbert, 1988). To the contrary, microcytosis (decreased MCV) is indicative of chronic disease (Abutarbush, 2015) as well as nutrition deficiencies. Furthermore, a diagnosis of iron deficiency anemia is very likely when microcytosis coexists with a decreased mean cell hemoglobin concentration (MCHC) (Green et al., 1997). On the other hand, an increase in the MCHC is observed in hemolytic anemia because of the released of hemoglobin from the ruptured erythrocytes in blood (Radostits et al., 2006).

Apart from the detection of reticulocytes and the presence of polychromasia, blood smear evaluation is needed in order to identify morphological abnormalities such as spherocytosis in case of both immune mediated hemolytic anemia and toxicity (Bundza et al., 1982). In diseases that cause physical injury to red blood cells, usually hemoparasitoses (Trypanosoma spp, Mycoplasma spp), schistocytes are observed (Gladden et al., 2016; Anosa et al., 1992). Moreover, oxidative damage of hemoglobin associated with onion toxicosis (Heidarpour et al., 2013) is denoted by the presence of Heinz bodies in the cytoplasm of red blood cells and/or eccentricocytes, where hemoglobin has been eccentrically accumulated (Borreli et al., 2009).

Finally, parasitic inclusions such Babesia spp, Theileria spp, Mycoplasma spp and Anaplasma spp (Gladden et al., 2016; Henniger et al., 2013; Izzo et
Detection of moderate to severe anemia and less sensitive in cases of mild anemia (Grace et al., 2007).

Other biological material testing

Fecal examination

Feces of anemic ruminants should be examined for color change due to the presence of blood, consistency, amount, shape, odor and the presence of mucous (Braun et al., 2008). Laboratory examination of feces includes microscopy for the detection of parasites, presence of occult blood and microbiology including molecular techniques for the detection of causative agents of the underlying disease (Bott et al., 2009).

Urinalysis

A strong indication for urinalysis in anemic ruminants is the red or dark brown color in urine. Urine sediment microscopy reveals the presence of red blood cells (hematuria). The red color of the supernatant after urine centrifugation either due to the presence of hemoglobin or myoglobin should be further differentiated by chemical analysis (Giannitti et al., 2014; Braun et al., 2008). Certain microorganisms can also be detected in urine employing either microscopy or molecular techniques (Denipitiya et al., 2017).

Bone marrow evaluation

Bone marrow evaluation is not routinely performed in ruminant medicine as it requires sample referral to a specialized laboratory. Bone marrow cytology is used to determine the regenerative response. If there is an increased cellularity in bone marrow, anemia is regenerative, while if there are few cells with morphological abnormalities, bone marrow dysfunction and dyserythropoiesis as well as non-regenerative anemia is more likely to occur (Fukunaka et al., 2010; Steffen et al., 1992). Bone marrow samples can be used for the detection of the causative agents of the underlying diseases.

Additional tests

Serological and molecular techniques are widely used for the etiological diagnosis of diseases that are associated with anemia in ruminants (Sonawane and Tripathi, 2015; Yang et al., 2015; Grova et al., 2011).
Moreover, chemical analysis is used for the detection of heavy metals and toxins in biological material as well as in feed and water (Keshavarzi, 2015).

CONCLUSION
Anemia is a frequent abnormal condition in ruminants. It is usually associated with pathological state of other tissues or organs rather than reflecting primary defects in erythropoiesis. A complete blood count is the indispensable part of the laboratory investigation of every case of anemia. However, a systematic approach includes history taking and clinical examinations followed by laboratory testing targeted to the clinical findings in order to identify possible etiologic factors.

CONFLICT OF INTEREST STATEMENT
The authors declare no conflict of interest.

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