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Review article
Ανασκόπηση

Diagnostic approach of anemia in ruminants

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Διαγνωστική προσέγγιση της αναιμίας στα μηρυκαστικά

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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

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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 εξωπαρασίτωση ή ενδοπαρασίτωση του γαστρεντερικού σωλήνα, στο σύνδρομο του αιμορραγικού εντέρου, σε έλκη του ηνύστρου, σε έμφραξη της οπίσθιας κοίλης φλέβας καθώς και σε αιμορραγία από το ουρογεννητικό 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 παράγονται από τα *Clostridium perfringens* τύπου D και *Clostridium hemolyticum* και η λεπτοσπείρωση. Επιπλέον, ορισμένα φυτά, φάρμακα ή βαρέα μέταλλα μπορούν να προκαλέσουν αιμόλυση των ερυθροκυττάρων καθώς και η έλλειψη ιχνοστοιχείων. Η ανοσολογικής αιτιολογίας αιμολυτική αναιμία έχει επίσης αναφερθεί στα μηρυκαστικά. Η μειωμένη παραγωγή ερυθροκυττάρων οφείλεται σε έλλειψη της βιταμίνης Β12 ή του σιδήρου, καθώς και σε χρόνια νοσήματα. Παθολογικές καταστάσεις του μυελού των οστών όπως η διήθηση από φλεγμονικά ή νεοπλασματικά κύτταρα όπως και η υποπλασία ή απλασία του μυελού των οστών σχετίζονται με μειωμένη παραγωγή ερυθροκυττάρων. 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. Από τις παραμέτρους της αιματολογικής εξέτασης, πέρα από τον αιματοκρίτη, η συγκέντρωση της αιμοσφαιρίνης, οι δείκτες των ερυθροκυττάρων μέσος όγκος ερυθροκυττάρων (Mean Corpuscular Volume, MCV), και μέση συγκέντρωση αιμοσφαιρίνης (Mean Corpuscular Hemoglobin Concentration, MCHC) συμβάλλουν στην ταξινόμηση της αναιμίας. Στο επίχρισμα του αίματος αξιολογείται το σχήμα και το μέγεθος των ερυθροκυττάρων, η παρουσία εγκλείστων, καθώς και η παρουσία δικτυοερυθροκυττάρων ενδεικτικών αναγεννητικής αναιμίας. Για τη διαφοροποίηση της αναιμίας σε αναγεννητική ή μη σπανιότερα γίνεται εξέταση μυελού των οστών. Ειδικότερα, η επιβεβαίωση ανοσολογικής αιτιολογίας αναιμίας στηρίζεται στην εξέταση Coomb's για την διαπίστωση αυτοαιμοσυγκόλλησης. Η απώλεια αίματος πέραν της αιμορραγίας διαπιστώνεται επίσης με την ανάλυση του ούρου και την κοπρανολογική εξέταση για την παρουσία αίματος ή/και παρασίτων. 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, parasitisism of the gastrointestinal track 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 causative 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 as 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 (Radostits 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 hemostatic disorders (Radostits 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.

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

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

*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

Hemolytic anemia

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 cattles (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 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

	Sheep	Goats	Cattle
Infectious Agents			
Babesia spp	A, Y	A	A, Y
Theileria spp	Y		A, Y
Anaplasma spp	A	A	A, Y
Trypanosoma spp	A, Y	A	A, Y
Mycoplasma spp	A		A
Bovine stephanofilarial dermatitis			A
Yellow lamb disease	Y		
Bacillary hemoglobinuria	A		A
Leptospira spp	Y		A
Toxic agents			
Allium spp	A	A	A, Y
Brassica spp			A
Ipomoa carnea	A		Y
Long-acting oxytetracycline	A		
Copper	Y	A	
Zinc	A		
Arsenic	A		A
Lead			A
Nutrition deficiency			
Copper	Y	Y	
Selenium	Y		
Phosphorus	Y		
Immune Mediated Conditions			
Vaccination			A, Y
Bovine colostrum fed to sheep	Y	Y	
IMHA			A, Y

*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 mediate 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 *Capripoxvirus* 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

	Sheep	Goats	Cattle
Nutrition deficiency anemia			
Cobalt/ folate	Y	A	
Iron	A, Y		A, Y
Anemia of chronic disease	A		A
Lumpy skin disease			A
Mycoplasma paratuberculosis	A		
Chronic toxicity			A, Y
Bluetongue	A		
Anemia secondary to bone marrow dysfunction or dysplasia			
Neoplasia			A
Bovine neonatal pancytopenia			Y

* 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

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

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

* 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.

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 (Deutskens 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).

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).

Symptoms of the underlying disease are also present and they can be either non-specific such as anorexia, fever and weight loss or specific to the certain disease.

The severity of clinical signs is negatively related to the chronicity of anemia and positively to the amount of blood loss. Therefore, chronic diseases as well as bone marrow insults are manifested by mild symptoms such as weight loss in adults and decrease of weight gain in lambs, reduction of foraging or

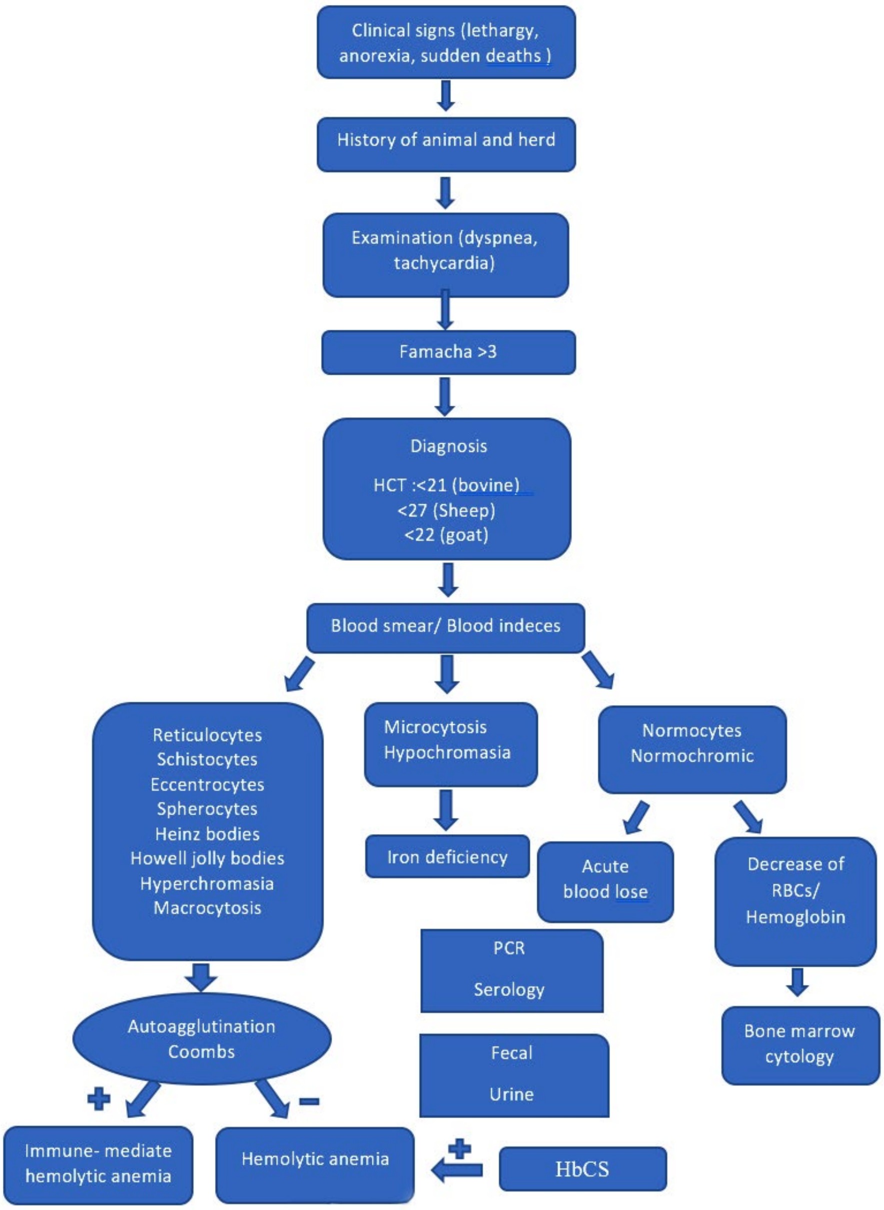

Figure 1. Algorithm for investigation of anemia in ruminants

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 haemonchonsis, in order to selectively administer antiparasitic compounds only in the infected and not all animals of an infected farm. This system is an easy to perform and of low cost method.

FAMACHA is based on the examination of the lower eyelid mucous membrane color against a laminated color chart presenting 5 color categories where 1 is the normal mucous membrane color and 5 is the white color. The score 3 is the borderline for starting medical treatment. The system has been validated using PCV as gold standard. The sensitivity was found to be higher in sheep than in goats negatively related to the cut off value of the PCV that was used to differentiate anemic from non-anemic animals (Sotomaior et al., 2012). Furthermore, conjunctiva color varies in different breeds therefore; modifications of the color in the chart based on breed specific colors are suggested (Moors and Gauly, 2009)

In bovine, FAMACHA was used to score anemia predominantly caused by *Trypanosoma congolense.* The system was found adequate to identify moderate and severely anemic cattle with a FAMACHA score of 4 and 5, respectively (Grace et al., 2007).

Despite the subjectivity of color estimate, FAMACHA remains a convenient rough estimate of anemia in ruminants prior to laboratory testing.

Laboratory evaluation

Blood testing

Complete blood count (CBC)

Clinical suspicion of anemia is confirmed by a complete blood count, mainly the hematocrit and red blood cell count. It is worth mentioning that in neonates, hematocrit value is relatively high at birth, while, till the age of 6 months, it decreases below the lower reference interval for adults. Different reference intervals are provided in literature reflecting differences in populations and equipment used for their determination. For instance, according to Schalm's hematology textbook (Weiss and Wardrop, 2010), hematocrit reference interval is 21-30%, 27-45% and 22-38% for cow, sheep and goat, respectively.

For the classification of anemia and the assessment of regenerative response, reticulocyte count along with blood smear evaluation should be performed. In regenerative anemia, the number of reticulocytes is 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 eccentrocytes, where hemoglobin has been eccentrically accumulated (Borreli et al., 2009).

Finally, parasitic inclusions such *Babesia* spp, *Theilleria* spp, *Mycoplasma* spp and *Anaplasma* spp (Gladden et al., 2016; Henniger et al., 2013; Izzo et al., 2010) can be found.

Alterations in the number and/or morphology of white blood cells are also observed in case of anemia and they are associated with the underlying disease. Similarly, anemia caused by hemostatic disorders can affect the number and/or morphology of platelets. For instance, thrombocytopenia has been reported in anemic calves with BVD-MD (Dabak et al., 2007).

Agglutination testing

Aggregation of erythrocytes forming irregular shapes is observed either in inflammatory reactions especially in bovine known as rouleaux formation (Weiss and Wardrop, 2010) or in autoimmune diseases where red blood cell clumps are not dissolved if blood is diluted with 0.9% saline (Nassir et al., 2011). Suspected immune- mediate hemolytic anemia is confirmed by direct Coombs' test which becomes positive when anti- immunoglobulin and anti- complement antibodies are mixed with host erythrocytes (Nassir et al., 2011). Both IgG and IgM reactive antibodies have been implicated in the pathogenesis of idiopathic immune-mediated hemolytic anemia, however, IgG is much more common than IgM. They are mostly considered as warm hemagglutinins since they are reactive at body temperature. Cold hemagglutinin disease (IgM antibodies that bind to erythrocytes below body temperature) results in thrombi formation that lead to ischemic necrosis usually observed at the tips of the ears.

While immune mediated anemia is usually regenerative, it can rarely be non-regenerative when bone marrow progenitor cells are targeted instead or along with mature erythrocytes.

Scoring of anemia without using a blood count analyzer

Efforts to estimate haemoglobin without the use of laboratory equipment and consumables have been made in human medicine and the Haemoglobin Colour Scale (HbCS) has been developed (WHO, 2004). The test requires a drop of blood that is applied on a special chromatographic paper which is compared to a laminated card with different colours related to different haemoglobin concentrations. The performance of this test in cattle was reported to be good for the 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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