Cytomorphological similarities between feline viral leukemia, bovine enzootic leukosis and adult T-cell leukemia/lymphoma: A review

Semelhanças citomorfológicas entre a leucemia viral felina, leucose enzoótica bovina e leucemia/linfoma de células T do adulto: Revisão de literatura

Similitudes citomorfológicas entre la leucemia viral felina, la leucosis bovina enzoótica y la leucemia/linfoma de células T adultas: Revisión de literatura

Júlia Meireles da Silva Silveira
ORCID: https://orcid.org/0000-0001-5553-1499
Universidade de Vassouras, Brazil
E-mail: juliameirelesss97@gmail.com
Sheila de Oliveira Medeiros
ORCID: https://orcid.org/0000-0001-5492-1532
Organização Pan-Americana da Saúde, Brazil
E-mail: sheila.omedeiros@gmail.com
Renata Fernandes Ferreira de Moraes
ORCID: https://orcid.org/0000-0001-7129-1587
Universidade de Vassouras, Brazil
E-mail: referreirauff@yahoo.com.br
Erica Cristina Rocha Roier
ORCID: https://orcid.org/0000-0002-1978-9254
Universidade de Vassouras, Brazil
E-mail: roier.erica@gmail.com
Bruna de Azevedo Baêta
ORCID: https://orcid.org/0000-0002-0172-556X
Universidade de Vassouras, Brazil
E-mail: babaeta@hotmail.com
Letícia Patrão de Macedo Gomes
ORCID: https://orcid.org/0000-0003-1373-9069
Universidade de Vassouras, Brazil
E-mail: leticiapatraomacedogomes@gmail.com
Gustavo Mendes Gomes
ORCID: https://orcid.org/0000-0002-4967-8029
Universidade de Vassouras, Brazil
E-mail: bigugomes@gmail.com
Ana Paula Abreu
ORCID: https://orcid.org/0000-0001-6385-3906
Universidade de Vassouras, Brazil
E-mail: ana_abreuvet@yahoo.com.br

Abstract
Leukemias are malignant neoplasms of hematological origin and originating from bone marrow cells. Innumerable species can be affected by this disease, which can be originated by several causes, including infection by viruses belonging to the *Retroviridae* family. In felines, humans and cattle, the leukemia-inducing retroviruses are Feline Leukemia Virus (FeLV), human T-cell lymphotropic virus type 1 (HTLV-1) and Bovine Leukosis Virus (BLV), respectively. In Brazil, the number of domestic cats infected with FeLV grows progressively, when compared to the incidence of infected animals in developed countries, such as the United States. In cattle, viral leukemia or enzootic bovine leukosis (EBL), caused by BLV, although asymptomatic, leads to decreased production and economic losses. In humans, HTLV-1 was the first human retrovirus described in the 1980s. In this work, the similarities between cytomorphological changes in felines, cattle and humans affected by FeLV, BLV and HTLV-1, respectively, were analyzed. The bibliographic findings showed that the affected species addressed share the presence of atypical and/or reactive lymphocytes, smudge cells, immature cells and nuclear cell atypias in peripheral blood.
Keywords: Morphological alteration; Blood smear; FeLV; HTLV-1; Leukemia; BLV.

Resumo
As leucemias são neoplasias malignas de origem hematológica e oriundas de células da medula óssea. Inúmeras espécies podem ser acometidas por tal enfermidade, a qual ser originada por diversas causas, incluindo a infecção por vírus

pertencentes à família *Retroviridae*. Em felinos, humanos e bovinos, os retrovírus indutores de leucemia são o Vírus da Leucemia Felina (FeLV), vírus linfotrópico de células T humano tipo 1 (HTLV-1) e Vírus da Leucose Bovina (VLB), respectivamente. No Brasil, o número de felinos domésticos infectados com o FeLV cresce de maneira progressiva, se comparado com a incidência de animais infectados em países desenvolvidos, como os Estados Unidos. Já em bovinos, a leucemia viral ou leucose enzoótica bovina (LEB), causada pelo VLB, apesar de assintomática, leva à diminuição na produção e perdas econômicas. Em seres humanos o HTLV-1 foi o primeiro retrovírus humano descrito na década de 80. Neste trabalho, as semelhanças entre as alterações citomorfológicas em felinos, bovinos e seres humanos acometidos pelo FeLV, VLB e HTLV-1, respectivamente, foram analisadas. Os achados bibliográficos mostraram que as espécies acometidas abordadas compartilham a presença de linfócitos atípicos e/ou reativos, smudge cells, células imaturas e atipias celulares nucleares em sangue periférico.
Palavras-chave: Alteração morfológica; Esfregaço sanguíneo; FeLV; HLTV-1; Leucemia; VLB.

Resumen
Las leucemias son neoplasias malignas de origen hematológico y originadas en células de la médula ósea. Innumerables especies pueden verse afectadas por esta enfermedad, que puede tener varias causas, entre ellas la infección por virus pertenecientes a la familia *Retroviridae*. En felinos, humanos y ganado, los retrovirus inductores de leucemia son el virus de la leucemia felina (FeLV), el virus linfotrópico de células T humanas tipo 1 (HTLV-1) y el virus de la leucosis bovina (VLB), respectivamente. En Brasil, el número de gatos domésticos infectados con FeLV crece progresivamente, en comparación con la incidencia de animales infectados en países desarrollados, como Estados Unidos. En bovinos, la leucemia viral o leucosis bovina enzoótica (LBE), causada por VLB, aunque asintomática, conduce a una disminución de la producción y pérdidas económicas. En humanos, HTLV-1 fue el primer retrovirus humano descrito en la década de 1980. En este trabajo se analizaron las similitudes entre cambios citomorfológicos en felinos, bovinos y humanos afectados por FeLV, VLB y HTLV-1, respectivamente. Los hallazgos bibliográficos mostraron que las especies afectadas abordadas comparten la presencia de linfocitos atípicos y/o reactivos, smudge cells, células inmaduras y atipias nucleares en sangre periférica.
Palabras clave: Alteración morfológica; Frotis de sangre; FeLV; Leucemia; HTLV-1; VLB.

1. Introduction

Leukemias belong to a group of clonal diseases derived from a single cell with genetic alteration in the bone marrow or in the peripheral lymphoid tissue, each type being determined by the specificity of the cell of origin affected (Almeida et al., 2017). The causes are varied, including viral infections. In felines, viral leukemia is a common infectious disease among cats and caused by the Feline Leukemia Virus (FeLV), which was first described in 1964 (Jarrett et al., 1964). In humans, adult T-cell leukemia/lymphoma is an aggressive neoplasm, and may be associated with human T-cell lymphotropic virus type 1 (HTLV-1) (Khanlari et al., 2018). Viral leukemia in cattle, on the other hand, is called enzootic bovine leukosis and is caused by the Bovine Leukosis Virus (BLV). Enzootic bovine leukosis is a limiting factor for the growth of cattle herds and causes great economic losses (Spadetto & Dias, 2013).

Hematology has great relevance in the identification of these diseases, especially through the changes found in blood cells. Morphological cell analysis is a key issue for abnormality identification and classification, early cancer detection, and dynamic changes analysis under specific environmental stress (Chen et al., 2012). It is known that changes in the shape of cells are related to part of the clinical condition of patients and are an important reflection of the stage of the pathophysiology of diseases, enabling better monitoring and, consequently, better prognosis for patients (Nowakowski et al., 2009; Chen et al., 2012; Mehta & Hoffbrand, 2014).

The present review aims to analyze and compare the cytomorphological similarities of felines, cattle and humans affected by leukemia of viral origin already reported in the literature. This comparison is important, since the advance in the understanding of the pathophysiology of the disease in one species can help to understand the same condition in the others, in order to enable its prevention and development of new treatments.

2. Methodology

The present work is a qualitative literature review. A literature review involves a critical evaluation identifying similarities and differences between existing literatures and the work being undertaken (Mweetwa, 2020). Literature review fosters a logical string of past relevant findings on a given topic that would whether corroborate, refute or create a new departure (Benacherine, 2019). Therefore, the topics developed and addressed were carried out through a bibliographic survey in books and journal articles (present in scientific information platforms, especially Pubmed). The articles were selected, firstly, based on their titles, keywords, relevance in the scientific world and publication date, prioritizing, above all, those published after 2010. Through each chosen article, involving only one of the three conditions addressed, the relevant information was selected separately. Such information was then compared with each other, so that it was possible to assess their similarities and include them in the present work, in order to reach the conclusions and synthesis of the ideas presented and written subsequently.

3. Diagnosis and Hematology of Feline, Bovine and Human Viral Leukemias

3.1 Felines

Retroviruses depend on an intermediate DNA for replication (Cunha, 2016). Thus, the single-stranded RNA genome undergoes the action of the reverse transcriptase enzyme, which translates the RNA into viral DNA. This copy of viral DNA is incorporated into the host genome (provirus), serving as a template for new viral particles that will be released through the cell membrane. For the production of proviruses, cellular DNA synthesis is necessary, which is why tissues with high mitotic activity, such as epithelium and bone marrow, are targets of the virus (Alves et al., 2015).

After exposure via oronasal, the feline leukemia virus is found, first, in the local lymphoid tissue, spreading, in a second moment, through lymphocytes and monocytes to the periphery, being able to infect the bone marrow during this primary viremia. After bone marrow infection, a secondary viremia can occur, with FeLV-containing leukocytes and platelets appearing in the blood (Little et al., 2020).

The diagnosis of feline leukemia induced by FeLV and its disorders is multifaceted, since, in addition to the importance of confirming FeLV infection, the concomitant diagnosis of secondary infections by pathogens, such as *Mycoplasma haemofelis*, is extremely important. In addition, according to Pereira et al. (2018), hematological knowledge is essential for understanding the pathogenesis of FeLV, assisting the veterinarian in making decisions before a feline infected by the virus.

Although FeLV was named after a tumor that first garnered its attention, most infected cats are presented to the veterinarian not for tumors but for anemia or immunosuppression (Hartmann, 2012). It is common to identify regenerative anemia (with or without thrombocytopenia), lymphopenia or neutropenia, and nucleated red blood cells in a blood smear from positive cats. The presence of these nucleated red blood cells is not certain of a regenerative anemia, and may be the result of a nonspecific spinal disorder, thus requiring a reticulocyte count for this determination (Alves et al., 2015).

Some morphological changes such as the presence of blasts (Ferreira et al., 2017), lymphoblasts (Valle et al., 2016), erythroid precursors (Shirani et al., 2011), toxic neutrophils (Almeida et al., 2019), smudge cells (Valle et al., 2016), large platelets (Almeida et al., 2019) and atypical lymphocytes (Ferreira et al., 2017) can occur more frequently. Some cells may also have a high nucleus/cytoplasm ratio (Cunha, 2016; Almeida et al., 2019).

The variability in viral recombination corresponds with variability in the pathogenesis and disease outcomes including aplastic anemia, T cell lymphoma/leukemia, myeloid leukemia, and immunosuppression (Weiss & Wardrop, 2010). In acute lymphoblastic leukemia (ALL), lymphoblasts are present in the blood and bone marrow and the same can occur with FeLV positive felines (Harvey, 2001; Thrall et al., 2015). However, in contrast to ALL, the vast majority of cats with chronic lymphocytic leukemia (CLL), according to Thrall et al. (2015), is negative for FeLV. Erythroid leukemia, on the other hand, is

commonly associated with underlying retroviral infections, particularly by FeLV, being uncommon in felines negative for the virus (Shirani et al., 2011; Cowell & Tyler, 2020). The complete blood count, as Little et al. (2020), should be performed every 6 months to monitor felines infected with FeLV, due to the frequency of hematological disorders present in animals with feline viral leukemia.

There are six stages in the pathogenesis of FeLV infection and the diagnostic methods for the virus can be influenced by these stages. According to Alves et al. (2015), the enzyme-linked immunosorbent assay (ELISA) and PCR (Polymerase Chain Reaction) are negative in the first stage, and the IFA (indirect immunofluorescence) becomes positive only from the fourth stage. The diagnosis of the infection is usually based on the detection of the soluble viral p27 antigen, through the use of rapid tests (PoC – Point of Care). Tests capable of detecting the FeLV antigen should not be performed from tears or saliva samples, since the reported sensitivities are low (Little, 2016; Westman et al., 2017; Little et al., 2020).

There are several ELISA kits that can be used in the clinical routine. Most results through these kits have high negative and positive predictive values and the test is not influenced by the presence of maternal antibodies or by vaccination against FeLV. Most cats will test positive within 30 days of exposure, although development of antigenemia can take longer in some cats (Little et al., 2020).

In infections classified as regressive, viremia and virus replication are retained before or immediately after the infection reaches the bone marrow. At the initial moment of infection, the virus replicates in mononuclear cells and, during that time, infected cats show positive results on the ELISA test. Meanwhile, in the infection classified as progressive, the immune response is insufficient in relation to the virus, causing an extensive viral replication, initially in the lymphoid tissues and, later, in the bone marrow (Hartmann, 2012).

Progressive and regressive infections can be distinguished through repetition of serological tests in peripheral blood; cats with regressive infection become negative at a maximum of 16 weeks post-infection, while cats with progressive infection will remain positive (Cunha, 2016). Initially, in both, there is the persistence of proviral DNA in the blood, detected by the PCR technique, but which are later associated with different viral loads when evaluated by quantitative PCR, where the regressive infection presents with low loads and the progressive one, with high virus loads (Hartmann, 2012).

As a positive screening test can definitely change the future of an animal considered positive, additional tests should be recommended, especially in low-risk and asymptomatic cats. Therefore, for confirmatory diagnosis, viral isolation tests are recommended, such as PCR (Gleich & Hartmann, 2009; Willett & Hosie, 2013; Alves et al., 2015). Sensitive PCR methods can detect provirus in the blood of cats with regressive infection that are antigen-negative (Hartmann, 2012).

IFA tests carried out on smears of peripheral blood or bone marrow are also available in some commercial laboratories for the diagnosis of feline viral leukemia. This assay can detect infection only after the blood cell precursors in the bone marrow are infected (stage 4), 6 to 8 weeks after exposure (Little, 2016). Therefore, when a result is positive, there is a probable indication that the animal is persistently infected.

3.2 Cattle

In all members of the *Retroviridae* family, the enzyme reverse transcriptase is present in virions, which synthesizes DNA from viral RNA, causing a chronic infection, since the host cell genome is integrated with proviral DNA. BLV is capable of infecting diverse populations of immune cells (Polat et al., 2017). However, the tumors that the virus induces usually originate from subpopulations of B cells, CD5+ and IgM+. The development of persistent lymphocytosis occurs through the reduction of the apoptotic process by CD5+ cells, the main population of leukocytes infected by the enzootic bovine leukosis virus (Pereira et al., 2013).

The clinical diagnosis of enzootic bovine leukosis is based on clinical manifestations (pneumonia, arthritis, mamitis or encephalitis) and epidemiological data (Pereira et al., 2013). According to Spadetto & Dias (2013), the diagnosis can be aided by clinical pathology. The blood test can reveal persistent lymphocytosis, suggesting infection with BLV; however, the absence of lymphocytosis does not exclude the possibility of infection (Pereira et al., 2013).

In addition, there may be morphological changes in cells in peripheral blood represented by smudge cells (Panei et al., 2013), atypical and neoplastic lymphocytes (Somura et al., 2014; Shaghayegh, 2019), Turk cells or plasmacytoid lymphocytes (Spinola et al., 2013) and immature cells (Khudhair et al., 2016; Oguma et al., 2017).

Marked lymphocytosis, involving normal-appearing small to mediumsized lymphocytes, is present in the blood of animals with chronic lymphocytic leukemia (Harvey, 2001). Persistent lymphocytosis with some normal-looking lymphocytes and some reactive lymphocytes can occur in animals with chronic viremia, of which persistent lymphocytosis found in cattle with bovine leukemia virus (BLV) infection is the most common. Persistent lymphocytosis is part of a continuous progression of BLV-infected animals that may eventually progress to a diagnosis of lymphocytic leukemia or lymphosarcoma (Thrall et al., 2015).

However, confirmation of the diagnosis is made only through laboratory methods. For indirect BLV diagnostic methods, particularly antibody-based tests, antibodies recognizing the p24 capsid protein encoded by the *gag* gene and the extracellular gp51 protein encoded by *env-gp51* are targeted (Polat et al., 2017). Some serological methods are able to identify antibodies in serum (Ruggiero et al., 2018), milk (Walsh et al., 2013) and in cell culture supernatants infected by the virus (Porta et al., 2019; Sato et al., 2019). The antibodies are located between 2 to 3 weeks after infection and remain detectable throughout the life of the host.

The test most used to diagnose enzootic bovine leukosis is the agar-gel immunodiffusion assay (AGID). The AGID is relatively inexpensive and is considered the official test for the diagnosis of the disease, since it is capable of simultaneously tracking several serum samples. However, as it is not a very sensitive test, it is inadequate for evaluating milk analyzes. Another serological test used in the diagnosis is the indirect or passive hemagglutination assay (PAH), in which viral glycoproteins are detected. However, its competence is sensitive to pH, temperature and trypsin (Polat et al., 2017). Radioimmunoassay (RIA) diagnosis is also used and has high sensitivity for detecting antibodies against the enzootic bovine leukosis virus (Pereira et al., 2013). Despite this, this test is inadequate to diagnose the virus en masse in herds and in recently exposed animals.

ELISA is more sensitive than the AGID test and, as stated by Nishimori et al. (2016), is able to identify specific antibodies to BLV in serum and milk samples. However, it requires a number of controls and can provide, in the early stage of infection, false-negative and false-positive results in calves with the presence of maternal antibodies (Nishimori et al., 2016; Polat et al., 2017). Still, according to Takeshima et al. (2016), the AGID, PAH and ELISA tests should not be used for testing calves less than 6 months old due to the presence of these antibodies of maternal origin.

The bovine enzootic leukosis virus is capable of integrating into disseminated regions within the host genome, remaining there even in the absence of detectable antibodies. Therefore, nucleic acid-based PCR methods can greatly accelerate the detection of BLV prevalence (Polat et al., 2017). Several genes present in the virus genome are aimed at verifying the prevalence of infection through direct PCR diagnostic methods, including the LTR region (Takeshima et al., 2016) and the *gag* (Yu et al., 2019), *pol* (Heenemann et al., 2012), *env* (Marawan, 2017) and *tax* genes (Somura, 2014).

Most PCR systems that detect the enzootic bovine leukosis virus has a nested PCR (nPCR) design (Polat et al., 2015, 2016). However, this method requires expensive real-time PCR machines and reagents, and involves difficult sample preparation protocols. In addition, false positive results can be obtained by contaminating the DNA. Recently, a new blood-based PCR system (PCR-DB) was developed, capable of amplifying the target regions of DNA without the need for isolation and

purification. PCR-DB method exhibited good reproducibility and excellent specificity and is suitable for screening of thousands of cattle, thus serving as a viable alternative to nested PCR and real-time PCR (Nishimori, 2016).

In addition, other diagnostic methods have already been described, such as Western blotting (Cuesta et al., 2018), syncytium induction assay (Sato et al., 2019; Watanuki et al., 2019) and detection of IFA antigens (Matsuura et al., 2019).

3.3 Human beings

Human T-cell lymphotropic virus type I (HTLV-I) is an encapsulated, positive-polarity, single-stranded RNA retrovirus belonging to the Retroviridae family (Melo et al., 2017). HTLV-1 was the first human retrovirus to be identified (Yoshida, 2005). ATL pathogenesis is not yet completely understood (Oliveira et al., 2016). After transmission, reverse transcriptase generates proviral DNA from genomic viral RNA, and the provirus is integrated into the host genome by viral integrase (Gonçalves et al., 2010). The virus is capable of multiplying in the host through mitotic division and virological synapses.

In this way, various components of the virus, including its RNA, are transferred across synapses from an infected cell to an uninfected cell. Inside the newly infected cell, viral RNA is transcribed into DNA. This newly transcribed DNA integrates with the cell's DNA, originating a newly infected clone. In the infected cell, through the second mechanism, HTLV-1 is able to induce mitotic division of the same, originating the other identical infected cells containing proviral DNA. In order to escape from the host immunity, HTLV-1 replicates as a provirus by increasing the number of infected host cells (Satou & Matsuoka, 2012).

The diagnosis of adult T-cell leukemia/lymphoma (ATL) is based on the combination of the clinical signs and malignant cellular morphological/immunophenotypic characteristics present, and on the confirmation of the presence of the human T-cell lymphotropic virus type 1 (HTLV-1), which can cause the disease. The hallmark of the disease is the presence of medium and/or large and atypical lymphocytes, with poly or multilobulated nuclei, with the appearance of a flower petal (flower cell), dense chromatin, absent or small nucleoli and expression of a CD3 +, CD4 +, CD5 +, CD7-, CD8- and CD25 + immunophenotype (Qayyum & Choi, 2014; Sibon et al., 2015; Oliveira et al., 2016; Melo et al., 2017; Hermine et al., 2018).

Flower cells can be seen peripheral blood smears. These cells are considered pathognomonic for ATL and allow diagnosis in isolation (Oliveira et al., 2016). As stated by Bazarbachi et al. (2011), the tumor cells of ATL can be detected in the peripheral blood or in the biopsy of affected organs. At least 5% of circulating abnormal T lymphocytes are required for a diagnosis of ATL in patients without histologically proven tumor lesions (Hermine et al., 2018).

HTLV-1 infection can result in adult T cell leukemia (Weiss & Wardrop, 2010). The non-singularity of the clinical laboratory manifestations of the disease led to its fragmentation according to the characteristics presented (Melo et al., 2017). ATL was then divided into four subtypes: indolent or smoldering (presence of less than 5% of atypical lymphocytes in peripheral blood and normal lymphocyte count), chronic form (presence of 5% or more of atypical lymphocytes in peripheral blood and lymphocytosis), lymphomatous form (lymphocyte count within the reference values and differential count showing 1% or less of atypical lymphocytes) and acute form (considered the most aggressive form and based on the presence of numerous atypical lymphocytes). In addition, some cases of ATL due to HTLV-1 infection can cause cytomorphological changes similar to CLL (Tageja et al., 2010; Oliveira et al., 2016).

A priori, to perform the screening, immunoenzymatic tests are used, such as ELISA, and agglutination, indirect tests, which seek to detect antibodies against HTLV-1, and may present false-positive reactions (Melo et al., 2017). Therefore, indirect immunofluorescence tests, radioimmunoprecipitation on polyacrylamide gel and Western blotting can be used as confirmatory tests. However, when they reach indeterminate results, there is a need to resort to more sensitive techniques, such as PCR (Bazarbachi et al., 2011; Melo et al., 2017) and Southern blot (Qayyum & Choi, 2014; Takatori et al., 2020). These techniques

are performed by a few laboratories and are therefore generally not accessible. However, they are not essential for diagnosis in most cases (Oliveira et al., 2016).

For the execution of the Southern blot (SBH) there is a need for a large amount of DNA without degradation and the method cannot be performed from formalin-fixed paraffin-embedded (FFPE) tissue samples. In the recent study by Takatori et al. (2020), a new diagnostic algorithm has been developed for the accurate identification of ATL using FFPE samples. The method had the combination of two assays capable of detecting the virus: the in situ hybridization of ultrasensitive RNA and quantitative PCR targeting the *tax* gene (*tax*-qPCR). The diagnostic algorithm that combines these two assays successfully evaluated 94% (112/119) of the samples and distinguished ATL from non-ATL cases, including HTLV-1 carriers with 100% sensitivity and specificity (Takatori et al., 2020). The authors hope that, in the future, this new method may replace SBH.

4. Cytomorphological Similarities Between Feline Viral Leukemia, Bovine Enzootic Leukosis and Adult T-cell Leukemia/lymphoma

Some similarities in the morphology of cells in peripheral blood can be found in the three species, since the diseases addressed can induce neoplastic formations and are caused by retroviruses (presence of the action of the reverse transcriptase enzyme and formation of the provirus), with tropism for lymphocites.

In the study by Ferreira et al. (2017), blood samples from 48 cats positive for feline leukemia virus were collected for hematological investigation, including cytomorphological analysis. It was possible to identify the presence of reactive or atypical lymphocytes in 27.1% of cats. According to the same authors, reactive lymphocytes are associated with antigenic stimulation, and can be constant findings in chronic antigenic stimulation related to peripheral lymph node hyperplasia in feline viral leukemia, for example. Atypical lymphocytes, on the other hand, are difficult to differentiate from reactive lymphocytes and suggest lymphoid neoplasia.

In cattle positive for the bovine leukosis virus, there are also reports of the presence of atypical lymphocytes (Tawfeeq et al., 2012, 2013; Somura et al., 2014; Khudhair et al., 2017; Shaghayegh, 2019) (Figure 1). According to Spinola et al. (2013), the presence of persistent lymphocytosis in cattle naturally infected by the bovine leukosis virus may be associated with an increase in atypical lymphocytes.

Figure 1. Photomicrograph of atypical monocytoid lymphocyte in bovine with EBL (enzootic bovine leukosis). 1000x magnification.
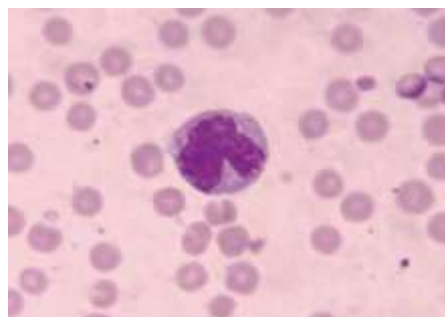

Source: Spinola et al. (2013).

Abnormal or atypical lymphocytes are also a possible finding in the peripheral blood of human patients infected with HTLV-1 (Figure 2). In the study by Khanlari et al. (2018), of 9 patients positive for ATL, 7 (78%) presented abnormal circulating lymphocytes and, in the research by Hodson et al. (2013), these lymphocytes were observed in the peripheral blood in 75% of

the patients. In the report by Nakamura et al. (2013), 44% of atypical lymphocytes were present in the blood smear of an infected patient.

Figure 2. Presence of abnormal lymphocyte in the peripheral blood smear of a human patient with ATL (adult T-cell leukemia/lymphoma). 1000x magnification.
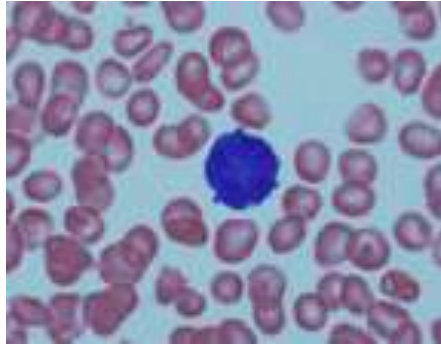
Source: Nakamura et al. (2013).

One of the pathognomonic atypias of ATL is the presence of flower cells in the blood smear of patients with the disease (Hodson et al., 2013; Graham et al., 2014; Qayyum & Choi, 2014; Sibon et al., 2015; Oliveira et al., 2016; Melo et al., 2017; Khanlari et al., 2018) (Figure 3). Flower cells are medium to large lymphocytes with coarse chromatin and a nucleus that resembles flower petals, which can be mistaken for smudge cells.

Figure 3. Flower cell in blood smear of human patient with ATL (adult T-cell leukemia/lymphoma). 1000x magnification.
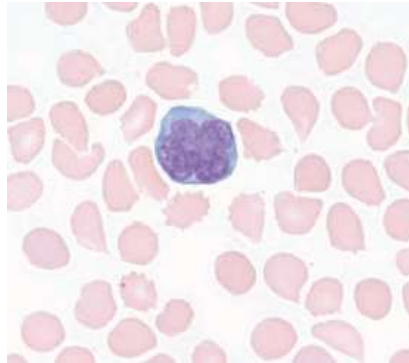
Source: Graham et al. (2014).

In addition, lymphocytes with cytoplasmic granulations have also been described in cattle (Spinola, 2010) and in humans (Hodson et al., 2013) (Figure 4) with viral leukemia.

Figure 4. Large granular lymphocyte in a blood smear from a human patient with ATL (adult T-cell leukemia / lymphoma). 1000x magnification.

Source: Hodson et al. (2013).

Another possible finding is smudge cells or Gumprecht shadows. Smudge cells are nuclei of free cells originating from disrupted nucleated cells. When cells rupture, the nuclear chromatin spreads out and stains eosinophilic (Cowell & Tyler, 2020). In the study by Panei et al. (2013), only bovines positive for BLV showed smudge cells in a peripheral blood smear analysis (Figure 5).

Figure 5. (A) Smudge cells (large arrows) in the peripheral blood of a bovine positive for BLV (bovine leukosis virus). (B) Normal lymphocyte in the peripheral blood of a bovine negative for BLV (bovine leukosis virus). 1000x magnification.
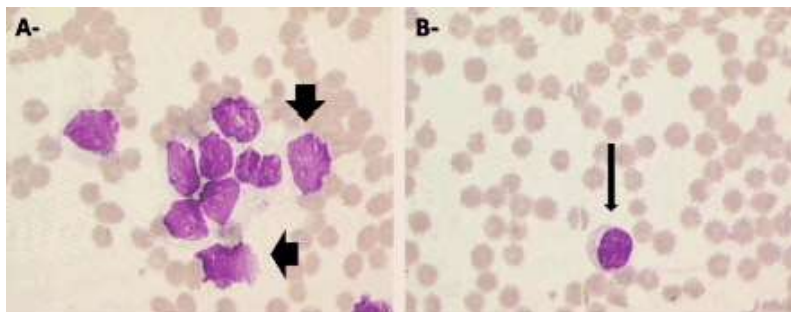
Source: Panei et al. (2013).

In B cells of humans with chronic lymphocytic leukemia, smudge cells are a common finding (Figure 6). In these patients, as proposed by the pioneering study by Nowakowski et al. (2009), the concentration of smudge cells is more than an artifact, since higher percentages of this finding are related to a lower life expectancy for these patients. In adult T-cell leukemia/lymphoma, some cells may also show atypical morphological patterns of chronic lymphocytic leukemia (Tageja et al., 2010; Oliveira et al., 2016). In the feline species, according to Almeida's research (2017), the fragility of the cells of some cats infected with FeLV can be accentuated, also inducing the frequent formation of smudge cells.

Figure 6. Smudge cells (arrows) on a peripheral blood smear in a human patient with chronic lymphocytic leukemia. 1000x magnification.
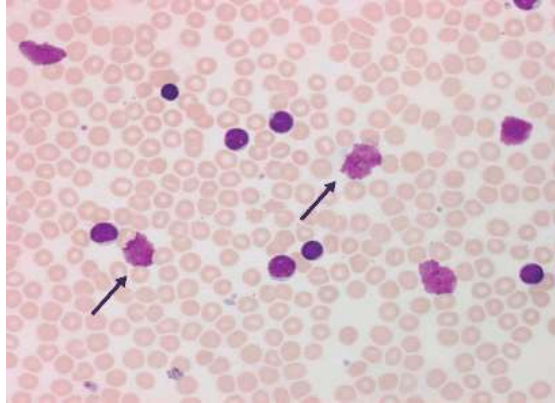

Source: Nowakowski (2009).

Morphologically immature cells can also be located in cats with feline viral leukemia (Shirani et al., 2011; Cunha, 2016; Valle, 2016; Ferreira et al., 2017) (Figures 7 and 8), in cattle with enzootic bovine leukosis (Spinola, 2010; Khudhair, 2016; Oguma et al., 2017) and in humans with ATL induced by HTLV-1 virus (Hodson et al., 2013; Qayyum & Choi, 2014; Oliveira et al., 2016; Rodríguez-Zúñiga et al., 2017).

Figure 7. Presence of a cell with lymphoid morphology (arrowhead) in a blood smear of a feline with acute lymphoblastic leukemia, infected with FeLV (feline leukemia virus). 1000x magnification.
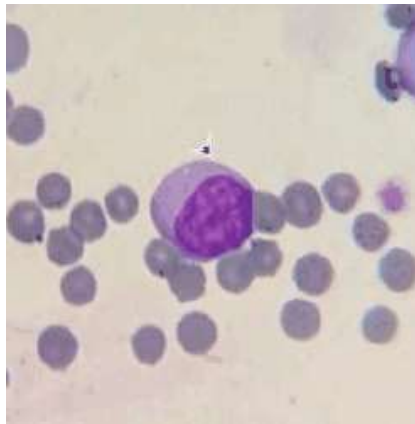
Source: Oliveira et al. (2020).

Figure 8. Erythroid precursors (arrows) and two metamielocytes (arrowheads) in the peripheral blood of a feline positive for FeLV (feline leukemia virus). 1000x magnification.
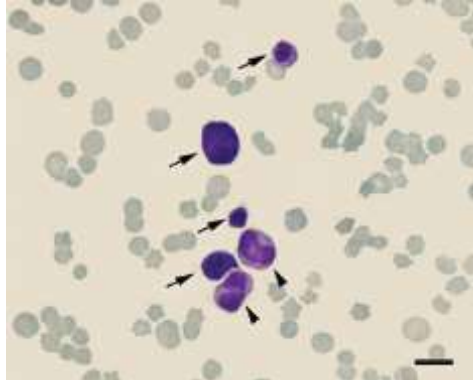

Source: Shirani et al. (2011).

In the study by Pereira et al. (2018), of 29 cats positive for FeLV and with clinical signs associated with the infection, 24.14% had neutrophils at various maturation periods (rods, myelocytes and metamielocytes). It is noteworthy that cats positive for feline viral leukemia and with clinical symptoms associated with the disease had more pronounced and frequent cytopenias. In that same study, immature lymphocytes were also found in a small percentage of cats (10.34%). In the report by Almeida et al. (2019), frequent cells of lymphoid origin with a lymphoblastic characteristic were also found in a feline infected by the feline leukemia virus. In cattle affected by EBL, there is a marked increase in lymphocytes (lymphocytosis), which are often not yet mature, and may be in the form of lymphoblasts.

In addition to the common presence of immature cells in the three diseases, the cell nucleus may also contain morphological atypias, such as a high nucleus/cytoplasm ratio in cases of feline viral leukemia (Almeida et al., 2019; Oliveira et al., 2020) (Figure 9), lymphocytes with double nucleus (Figure 10), or deformed, rhiniform, marked, pycnotic and condensed nuclei in cattle with EBL, and, in humans, hyperchromatic nuclear chromatin and deeply lobed nuclear contours characteristic of the flower cells previously addressed (Khanlari et al., 2018). Hodson et al. (2013) observed, as the most frequent characteristics in their research, irregular or convoluted nuclei (Figure 11) and bilobed nuclei (Figure 12). Mitotic figures can also be observed in felines (Almeida et al., 2019) and in infected cattle (Spinola, 2010).

Figure 9. Lymphocyte with an exuberant cell nucleus in relation to cytoplasm (N) in a blood smear of a feline with acute lymphoblastic leukemia and infected with FeLV (feline leukemia virus). 1000x magnification.
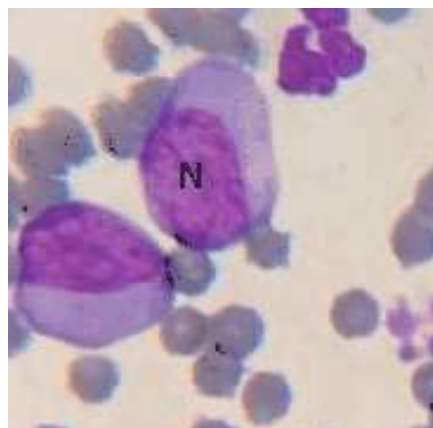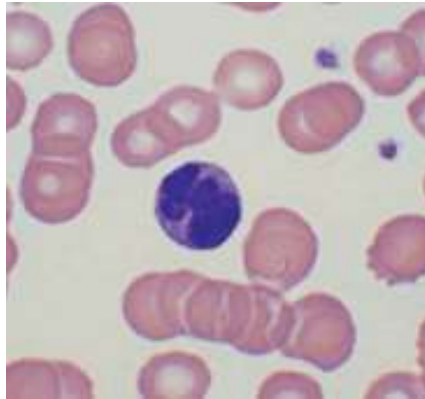

Source: Oliveira et al. (2020).

Figure 10. Photomicrograph of an atypical lymphocyte with double nucleus in a bovine positive for BLV (bovine leukosis virus). 1000x magnification.
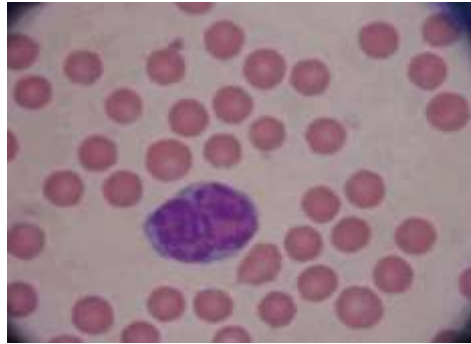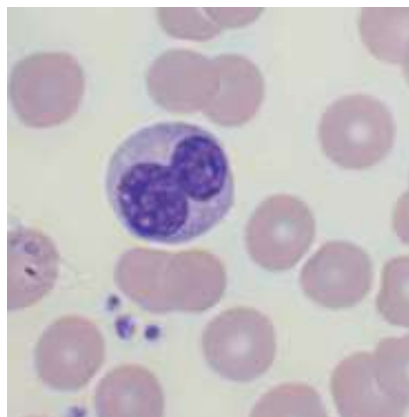

Source: Spinola et al. (2013).

Figure 11. Lymphocyte with convoluted nucleus in a blood smear of a human patient with ATL (adult T-cell leukemia/lymphoma). 1000x magnification.

Source: Hodson et al. (2013).

Figure 12. Lymphocyte with bilobed nucleus in a blood smear of a human patient with ATL (adult T-cell leukemia/lymphoma). 1000x magnification.

Source: Hodson et al. (2013).

5. Final Considerations

As a result of the present review, it was possible to conclude that the hematological findings of leukemia in the studied species showed great similarity among themselves, mainly due to the presence of immature cells in peripheral blood, atypical and/or reactive lymphocytes, smudge cells and nuclear atypias.

12

Therefore, knowledge about the common morphological changes between species can help the clinician in the diagnosis and prognosis of leukemias of viral origin. Such similarities may be the target for advances in the treatment of cattle, felines and, still, humans with leukemias caused by retroviruses.

In addition, cytomorphological changes, even when analyzed in isolation, are enough to raise suspicion about leukemia. Therefore, knowledge related to the alterations most commonly found in the peripheral blood cells of patients affected by this disease is of paramount importance. However, in animal species, such as feline and bovine, the methods of diagnosis of different types of leukemia are not easily available as in cases of leukemia in human beings.

Therefore, through the information involving the cytomorphological characteristics of viral leukemias addressed in the topics of this literature review, it is concluded that the continuous study of these characteristics will contribute, then, to greater future knowledge related to the detection of viral leukemias, consequently helping in the earlier and more effective diagnosis and monitoring of felines, cattle and humans affected by this pathology. For this reason, further studies in the area in question are extremely important.

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