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# **CANINE TRANSMISSIBLE VENEREAL TUMOR: CYTOMORPHOLOGY AND MODULATION ASPECTS OF PRO-INFLAMMATORY TUMOR MICROENVIRONMENT BY HIRUDOTHERAPY**

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## ***Abstract***

*In this paper, which is an integral part of a bachelor degree, we describe morphological and cytomorphological aspects of the canine transmissible venereal tumor, which are presented in the relevant literature up to this point. There were outlined the cytomorphological categories and their importance in defining a correct and complete therapeutic regimen for this tumor. There were 5 cases presented, in which the Sticker tumor had different localisations, different levels of violence, and diverse matters of the microenvironment. Also, Hirudotherapy was proposed as a complementary therapy to chemotherapy, but it was also proposed as a single therapy to be used, because of its antiinflammatory, anti-tumoral and immunostimulant effects. The evolution for each case was tracked weekly. The results showed the potential of this kind of complementary therapy in treating the plamocytic forms of the canine transmissible venereal tumor.*

**Keywords:** *canine transmissible venereal tumor (CTVT), cytomorphological characterization, tumor microenvironment, hirudotherapy*

## ***Introduction***

Canine transmissible venereal tumor (CTVT), also known as transmissible venereal tumor (TVT) or Sticker's sarcoma, is a transmissible neoplastic process affecting dogs. CTVT is spread by the transfer of live cancer cells between dogs, usually during mating. CTVT causes tumours that are usually associated with the external genitalia in both male and female dogs.

CTVT also has extragenital locations such as skin, subcutaneous connective tissue, oral and nasal cavity [3], musculature, mammary gland or rectum [4, 5].

The progression of this tumour is unique in that it follows a predictable growth pattern. In natural and experimental cases, the growth pattern includes a progressive growth phase, a plateau phase, and a regression

phase, the latter phase being followed by the onset of "transplant immunity" in immunocompetent adults, whereas metastases occur only in immunosuppressed dogs and puppies [1].

Because of the unique nature of CTVT transmission and progression, experimental investigations of various aspects of CTVT biology have been used to provide clues to the immunobiology of tumours in both animals and humans.

Clinical history, signaling, and cytologic features are often evident to establish the diagnosis, although biopsy and histologic examination may be required in atypical cases [2].

CTVT belongs to the category of round cell tumours with oval or round nuclei, with one or more eccentrically located nucleoli, granular or coarse chromatin, and cytoplasm is weakly basophilic, with perinuclear optically hollow vacuoles. In view of these cytomorphological aspects, the tumour is classified into 3 types respectively, lymphoid type, plasma cell type and mixed type [3]. In addition to the cytomorphological aspect, the tumour microenvironment is very important as it profoundly influences the behaviour of immune cells that interact dynamically with tumour cells and tumour stroma, thus playing an important role in disease progression.

In recent years, neutrophil biology in the context of cancer has seen a new resurgence. Emerging data show that neutrophils are far from being a simple homogeneous population, with literature attesting that they participate in tumour progression, acting both in primary tumours and in the (pre)metastatic niche. In primary tumours, neutrophils are involved in mediating angiogenesis and tumour cell proliferation, and through several different factors, individually or in combination, they increase vascular permeability and thus tumour cell extravasation and metastasis [6].

Inhibition of the migration of pro-inflammatory neutrophils from the tumour microenvironment by the application of *Hirudo medicinalis* leeches in the peritumoural area is a complementary therapy which, in this paper, I wish to associate with classical allopathic therapy, trying to thus to inhibit tumour progression and metastasis.

### ***Materials and methods***

The study was conducted on 5 canine animals, aged between 2 and 13 years, 2 females and 3 males, from different geographical areas, diagnosed with transmissible canine venereal tumor, located genitally in 60% of cases and extragenitally in 40% of cases.

**Table 1 Epidemiological details of the cases**

Crt. no.	Geographical area	Age	Sex	Anatomical location
1.	rural	11 years	male	peniană
2.	suburban	13 years	female	vulvară
3.	urban	9 years	female	vulvară
4.	urban	5 years	male	nazală și orală
5.	suburban	2 years	male	nazală

In the animals taken in the study, a cytomorphological examination was performed by scraping the tumor formation followed by smear examination, staining and microscopic examination. Smears were stained using May-Grunwald Giemsa panoptic stain. The principle of this method is based on the successive use of neutral stains and fixative in a mixture. We used this technique to characterize, cytomorphologically, the typology of the tumor formation and the tumor microenvironment (7).

Also, in the study we combined classical therapy of venereal tumours with hirudotherapy. Leech therapy is not as simple in application as it might seem at first. After the treatment, if it is performed correctly, no complications occur, the essential condition being to select the correct leech application points.

Depending on the place chosen for the application of leeches, one effect or another on the animal body is ensured, therefore, the choice of methodology in hirudotherapy is one of the main factors for a lasting therapeutic treatment.



**Figure 1 Application of perivulvar leeches for more effective local and regional action**

Essential in the application of leeches is to clean the application area, avoiding the use of any smelly disinfectant. The leech is grasped, with fingers, by the head, applied to the skin and waited until it catches. A leech, during therapy, can extract around 3-10 ml of blood and introduce 8-10 ml of saliva into the blood (8).

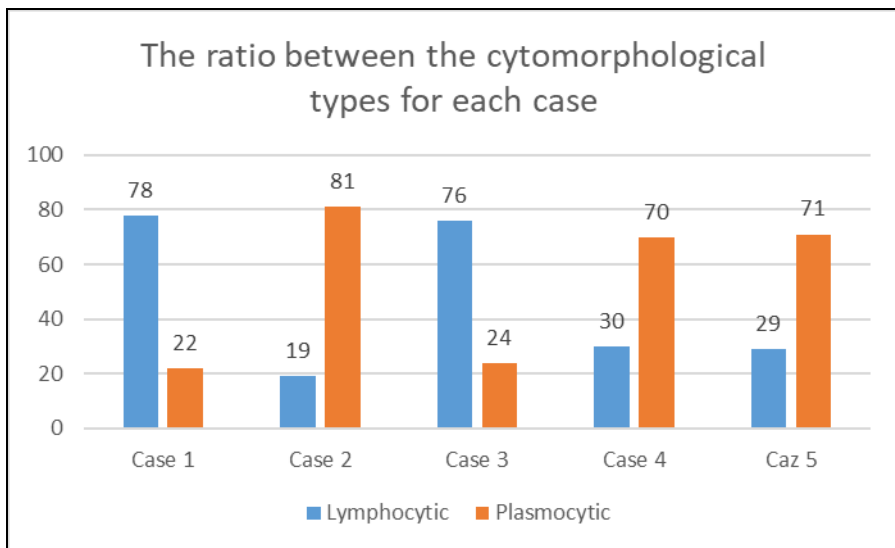
## RESULTS AND DISCUSSION

The tumoral scrapings showed the cytomorphological aspects of the masses, which helped classifying the neoplasm in the respective cytomorphological category: plasmocytic, lymphocytic or mixt, and to the description of the tumoral microenvironment.

There were 3 cases framed into the plasmocytic category and 2 cases framed into the lymphocytic category. This matter constituted a very important factor in the establishing of the treatment regimen: simple or combined.

**Table 2 Cytomorphological types of CTVT found in the study**

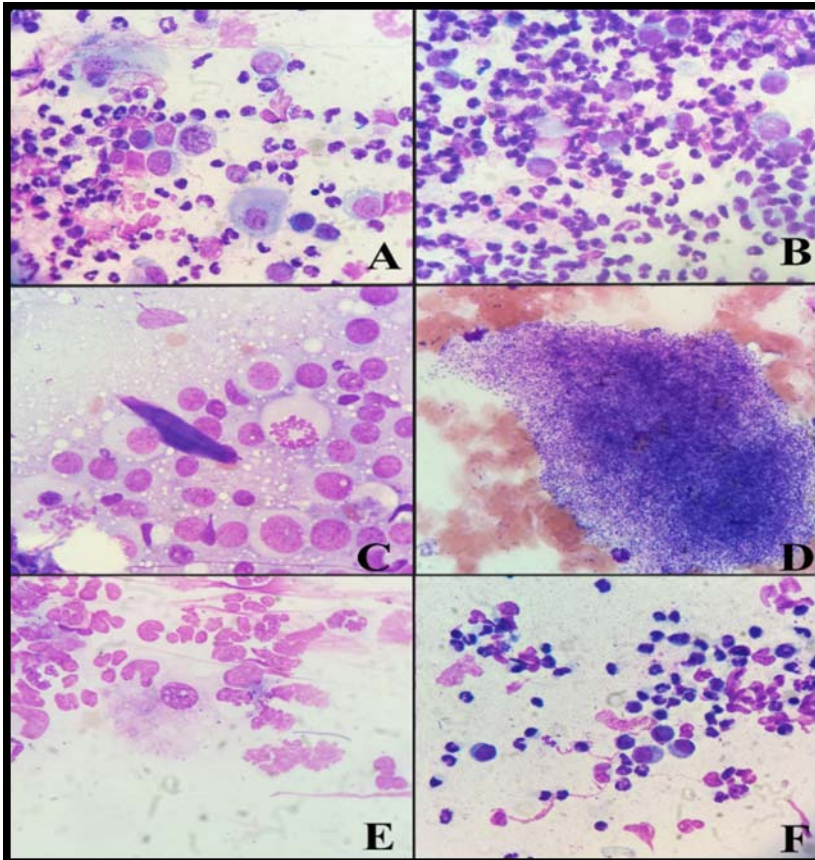
Case no.	Case 1	Case 2	Case 3	Case 4	Case 5
Cytomorphological type	lymphocytic	plasmocytic	lymphocytic	plasmocytic	plasmocytic
Cell ratio	78L:22P	81P:19L	76L:24P	70P:30L	71P:29L



**Figure 2 The ratio between the cytomorphological types for each case**



The most violent cases were represented by the tumor masses which were framed in the plasmocytic cytomorphologic category. They had an invasive character and presented with resistance to simple chemotherapeutic therapy. In these cases, the combined therapeutic regimen was used, with hirudotherapy complementary to chemotherapy. In contrast, for the cases which were framed into the lymphocytic cytomorphological category, the elected therapy for treating the tumor masses was simple hirudotherapy.



**Figure 3** In A and B degenerated and aged inflammatory cells with multilobulated nucleus (neutrophils), which signal the presence of a septic inflammatory process. A. Leukocytes (lower right corner), giant cell – macrophage, which phagocitated 4 neutrophils (upper left corner) and the presence of an atypical mitosis in the middle. B. Dominantly aged and degenerated inflammatory cells, typical for a septic inflammatory process. C. Keratinocyte, an element which can be seen when the scraping was done from the proximity of the cutaneous tissue and an atypical mitosis. D. Massive bacterial colony. E. Macrophage in the tumoral microenvironment. F. Lymphocytes in the tumoral microenvironment.

Another important aspect is represented by the entirety of elements which are constituting the pro-inflammatory tumoral factors. The chronicization of the inflammatory process slows or prevents the withdrawal of the neoplasm under treatment.

The microenvironment is represented by the entirety of elements present at a microscopic level in the tumor. Regular cytologic reexamination can offer important information concerning the evolution of the tumoral mass and the efficacy of the therapeutical regimen used.

The cases which were taken into discussion were cytologically tracked. Each week, tumoral scrapings were taken for the microscopic examination. These periodical reexaminations gave us informations about the tumoral stage of development or remission, inflammatory cell population and bacterial population, depending on the case.



*Figure 4 Case 4, Geronimo, CTVT with nasal and oral localization, before treatment*

In 3 of the 5 cases, in the first 2 weeks of treatment, a significant reduction of the inflammatory cells was noticed, as well as a reduction in the number of atypical mitotic figures.



*Figure 5 the evolution of the nasal tumor in the 3rd week of treatment*



*Figure 6 Evolution of the oral tumor in the 3rd week of treatment*

In the first 2 weeks, in cases 2 and 4, which has an overgrown bacterial infection located at tumor level, a substantial decrease in bacterial elements, up to disappearance, could be seen at the cytological reevaluation.

A specific cytological characteristic which signals the beginning of the tumoral retraction is the partial or total reduction of the tumoral mitotic

indicator. It loses its invasive and metastatic character, aspects which enable the organism and the treatment to fight more efficiently against the tumor.



*Figure 7 Case 4, Geronimo, at the end of the treatment*



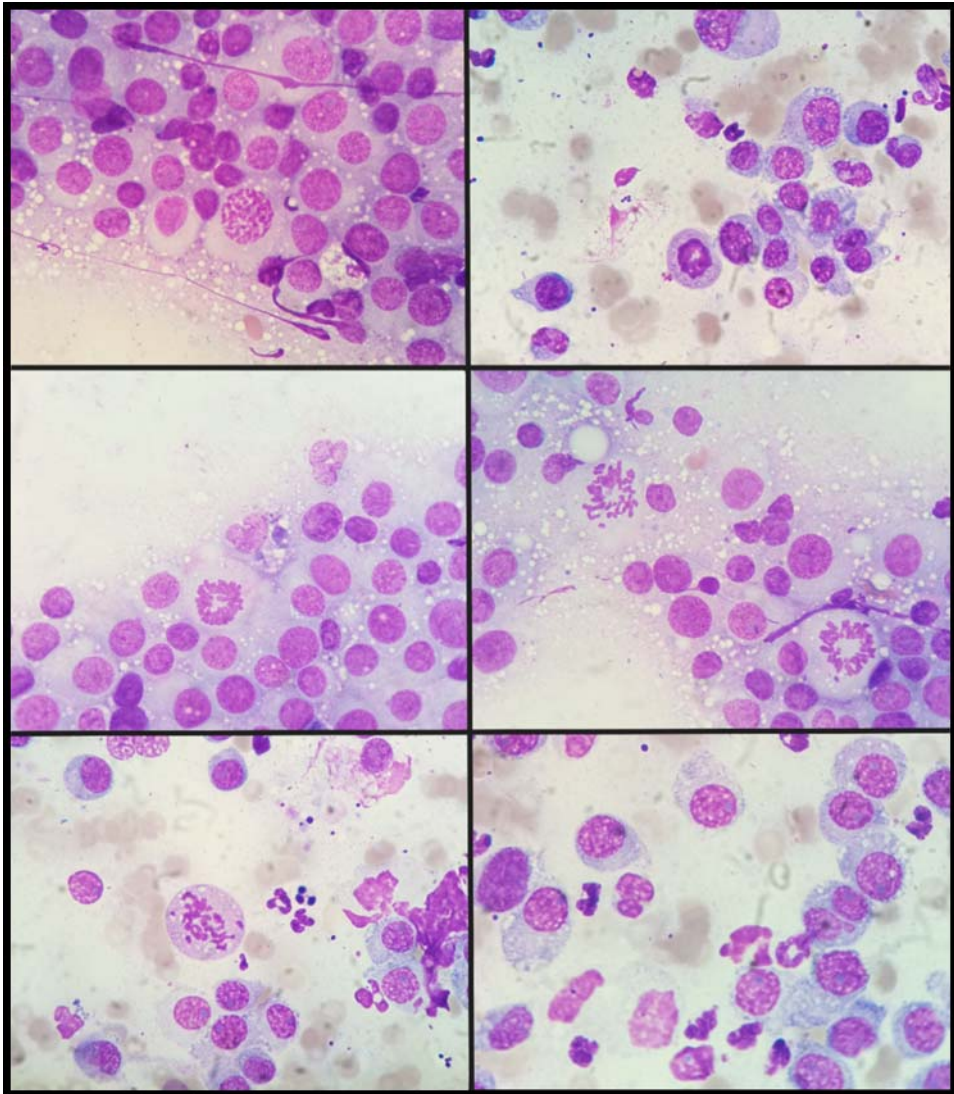
*Figure 8 Case 2, Baba, before the treatment*



*Figure 9 The tumor mass in the 4th week of treatment*

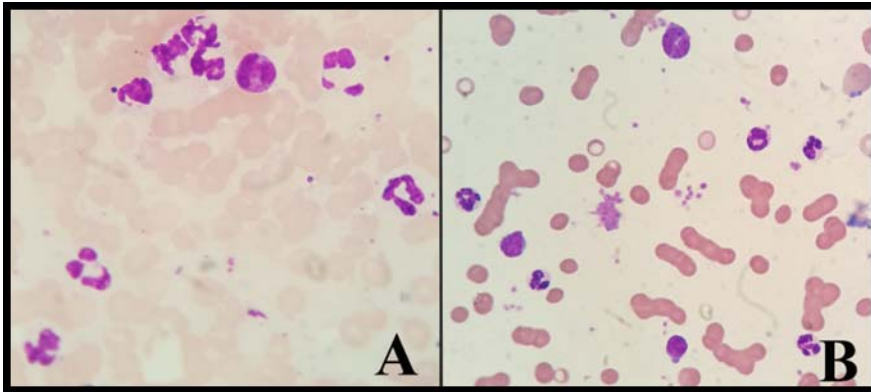


*Figure 10 Case 2, Baba, the vulva at the end of the treatment*



*Figure 11 CTVT cells in different mitosis stages*

The leukocyte formula represents the percentage and the ratio between the leukocyte categories from the blood or from other internal body fluids. In this study, we used blood samples for performing this test.



**Figure 12** *Leukocytes at the microscope. A. Neutrophils with multilobulated nucleus. Degenerated neutrophils. The presence of an eosinophil. B. Neutrophils with multilobulated nucleus. The presence of a monocyte. Presence of lymphocytes.*

**Table 3** *Leukocyte formulas before the treatment*

Leukocyte category	Case no.	Case 1	Case 2	Case 3	Case 4	Case 5
Neutrophils		81	90	76	80	87
Eozinophils		3	2	4	4	8
Bazophils		0	1	0	0	0
Lymphocytes		12	5	16	11	4
Monocyte		4	3	4	5	1
<b>Total</b>		<b>100</b>	<b>100</b>	<b>100</b>	<b>100</b>	<b>100</b>
<b>% neutrophils</b>		<b>80%</b>	<b>90%</b>	<b>76%</b>	<b>80%</b>	<b>87%</b>

The values presented in Table 3, showed a significant reduction in neutrophils between weeks 2-5, as it will be illustrated in Table 4.

**Table 4** *Percentile development of neutrophils from the leukocyte formula along treatment weeks 1-5*

Case no.	Week	Week 1	Week 3	Week 5
Case 1		80%	76%	68%
Case 2		90%	81%	72%
Case 3		73%	69%	-
Case 4		74%	70%	67%
Case 5		80%	-	-

There can be noted drops of the neutrophils percentage, which have been directly correlated with the start of the tumoral remission period. This emphasizes the anti-inflammatory effect of the leech saliva, as well as the complementary potential of hirudotherapy in this context.

### ***Conclusions and recommendations***

1. Canine transmissible venereal tumor is a neoplastic formation of clonal origin, which transmitted by transplantation from one individual to another both genitally and extragenital;
2. According to the literature, the age range in which canine transmissible venereal tumor is most common is 2-5 years. In our study, the age of the affected animals was between 2-13 years, 3 of them were older than 5 years, namely: 9, 11 and 13;
3. In our case report, the localization of CTVT was found at the genital level in 60% of them, in the vulva, penis and foreskin, as well as extragenitally in 40% were found nasally and orally;
4. Once the cytological examination has been carried out in order to make a diagnosis, the following must also be established the cytomorphological category of the neoplasm. This is very important for establishing the therapeutic protocol to be instituted;
5. Cytomorphologically, 60% of venereal tumours microscopically examined were classified as PLASMOCYTIC and 40% as LYMPHOCYTIC. In our case report, we did not encounter the mixed type.
6. Tumour microenvironment is a very important index to understanding tumor progression. The most important element of the tumour microenvironment followed in this study, a was the presence of pro-inflammatory cells, their presence marking a reserved prognosis.
7. Proposed therapeutic regimens for the treatment of transmissible canine venereal tumor have were:
  - a. Vincristine (0.7 mg/m<sup>2</sup>) + Hirudotherapy for cases where category cytomorphological category was plasmacytic. Cases 2, 4 and 5.
  - b. Hirudotherapy for cases where the cytomorphological category was lymphocytic. Cases 1 and 3.
8. In case 2, after single therapy with Vincristin proved ineffective in treating the tumour, after a 5-week break, it was used in conjunction with hirudotherapy as a method complementary therapy. After 4 weeks of this therapy regimen, the formation tumour went into remission.



9. Evaluation of the effectiveness of the therapy was performed by assessing the tumour volume at the macroscopically, and by microscopic assessment of the cellularity of the tumour microenvironment and tumour proliferation index.
10. Hirudotherapy has proven to be an effective complementary method in treating tumours in cytomorphological plasmacytic cases, but also as a method of unique treatment in lymphocytic cytomorphological cases. This is due to the anti-inflammatory and anti-tumour properties of leech saliva.

### ***Acknowledgement***

This study is part of an undergraduate research carried out in the Faculty of Veterinary Medicine, *Spiru Haret* University, 2023.

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# STUDY ON THE MORPHO-PATHOLOGICAL CHANGES INDUCED BY THE AFRICAN SWINE FEVER VIRUS

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## **Abstract**

*African swine fever (ASF), also called Montgomery's disease, is a highly contagious, non-zoonotic, viral infectious disease with morbidity and mortality of 98-100% especially in free areas where the pig population is most susceptible. Due to the serious and rapid potential for the spread of the disease and the far-reaching socio-economic consequences for international trade in animal products, ASF has been classified as a List A disease by the World Organization for Animal Health (OIE). From a clinical perspective, ASF may have similarities with several other pig diseases, mainly classical swine fever and porcine reproductive and respiratory syndrome (PRRS). Therefore, the morpho-pathological lesions detected in the 98 cases studied were corroborated with laboratory investigations to establish the diagnosis certainty.*

**Keywords:** *african swine fever virus, clinical signs, morpho-pathological lesions, polymerase chain reaction, enzyme-linked immunosorbent assay*

## **Introduction**

African swine fever (ASF) is an exceptionally lethal haemorrhagic viral disease affecting domestic pigs and Eurasian wild boars [1], irrespective of age and maintenance status, evolving with superacute and acute forms in new outbreaks of the disease and with subacute, chronic to atypical forms in old outbreaks.

ASF was first described in Kenya in 1921, and since 2016 a significant increase in the number of outbreaks has been reported, including in Europe [6, 7].

In Romania, the disease was diagnosed for the first time in 2017, at the The Institute for Diagnosis and Animal Health (IDAH), in two pigs from a household with 5 pigs (1 dead and 4 live), located on the outskirts of Satu Mare, close to the border with Hungary and Ukraine. A second outbreak, secondary of the first, was confirmed one day later, in a household with 4 pigs (1 dead pig and 3 sows without clinical signs) located less than 1000

meters away from the first infected household. Currently, The National Sanitary Veterinary and Food Safety Authority reported that, nationally, only 10 ASF outbreaks are still active [8].

The causative agent of the disease is a large, complex, enveloped DNA virus belonging to the family *Asfarviridae* and the genus *Asfivirus* and is known to be exceptionally stable in the environment, being detectable in pig tissues for months or even years at low temperatures [2]. The main routes of transmission of ASFV are direct contact between susceptible species and indirect contact caused by consumption of contaminated feed, vehicles, habitat, pasture, visitors, caretakers, traders, veterinarians, but also by arthropod vectors (mites) [1, 3, 4, 5].

The clinical signs of the disease, in wild boar and domestic pigs, vary considerably, from fatal to subclinical, depending on the immunity of the individual and the virulence of the strain involved and are represented by presymptomatic hyperthermia, respiratory, digestive and nervous disorders and from a lesional point of view, the predominant are the haemorrhagic circulatory changes [8].

The epidemiological situation of ASF, in the Member States and worldwide, presents an increased risk of further spread of this disease in the territory of the European Union [11].

In the absence of an effective vaccine against ASFV, preventing the spread of the disease in free countries depends on the implementation of strict rules of epidemiological surveillance, animal identification and traceability, biosecurity on farms and during the movement of pigs or pork products as well as of import policies [9].

This included the need for ASF to be considered a disease requiring appropriate risk management through the development and refinement of national control programmes and the need to recognise risk communication as an essential component to effectively address high-risk pathways and practices. Countries were also encouraged to maintain transparency through timely notification of diseases and manage risk through the implementation of international standards in relation to zoning, compartmentalization and the application of commodity-based trade measures.

At the national level, the National Veterinary Sanitary and Food Safety Authority has issued an *Operational manual for the intervention in outbreaks African swine fever* which aims to describe, step by step, the way for implementation of control measures, starting from the notification of suspected disease to disease control measures in outbreaks [10].

## **Materials and methods**

The study was carried out between January 2019 - April 2023, in the laboratory of The Veterinary and food safety division (DSVSA), Prahova and includes a total of 98 domestic pigs from households in which the ASF diagnosis was confirmed.

The pigs in the study were both live animals and cadavers with a specific clinical and/or lesional signs of ASF and in which ASFV was subsequently confirmed, thus complying with Commission Decision 2003/422/CE approving the *Diagnostic Manual for African swine fever*, which states that the diagnosis of ASFV shall be made in accordance with the procedures, sampling methods and criteria for the evaluation of the results of laboratory tests described in the manual and which are based on:

- (a) detection of clinical signs and post-mortem lesions of the disease;
- (b) detection of virus, antigen or genome in samples of tissues, organs, blood or faeces of pigs;
- (c) demonstration of a specific antibody response in blood samples.

In the present study, the diagnosis of ASF certainty was made taking into account the requirements of the manual, as follows:

(a) in live animals, suspected of ASF, we identified the macroscopic lesions present, and subsequently collected blood, without EDTA, for detection of anti-AFSV antibodies; in cadavers, after post-mortem examination, we collected organ fragments from spleen, kidney, lung, lymph node, heart and/or blood, for the identification of viral DNA;

(b) virus detection was performed by the Real Time PCR technique using the "High Pure RNA Isolation Kit" and,

(c) detection of anti-ASFV antibodies was performed by the indirect immunoenzymatic technique (ELISA) using the multi-antigen indirect ELISA kit for the detection of antibodies against P32, P62 and P72 of the ASF virus in porcine serum, plasma or blood filter paper samples.

## ***Results and discussion***

Clinically, ASF may have similarities with several other swine diseases, mainly with classical swine fever and porcine reproductive and respiratory syndrome (PRRS), so laboratory investigations are the only way to make a definitive diagnosis. Furthermore, the clinical signs of ASF may vary depending on the pathogenicity of the strain, the infective dose, the route of infection [5] and the clinical evolution may range from overactive forms to subclinical or inapparent infections. The virus has a particular affinity for

cells of reticuloendothelial system and for leukocytes, in which it multiplies rapidly and consequently induces gross lesions. The lesions are the result of direct action of the virus or are caused by the deposition of Ag-Ac complexes, generating areas of necrosis followed by haemorrhages [12].

In our case, the animals developed a lesional picture characteristic of the acute form of the disease, the lesions detected being exemplified by pictures. Thus, on the skin we detected a pronounced exanthema, generalized or localized, of light red or purplish red colour. Against the background of hyperemic or cyanotic areas (fig 1), haemorrhages of various sizes (spots, bruises, effusions) are very often observed. Sometimes edema of the ear pavilions is also observed (fig. 2).



*Fig. 1 Ecchymoses and petesis on the ventral side of the body*



*Fig. 2 Edema and cyanosis of the left auricle*

In the abdominal cavity we detected a clear, slightly yellowish or bloody, depending on the case, ascitic fluid and the lymph nodes, especially the mesenteric ones, showed pronounced haemorrhages (Fig. 3). The mucosa of the digestive tract is inflamed, congested, with haemorrhages of variable size. Superficial necrosis and ulcers of the mucosa of the small intestine are also found. The wall of the cecum is intensely edematous, with numerous haemorrhages on the mucosa, with much thickened folds resembling cerebral circumvolutions ( fig. 4).

At the gastric level, we detected a sero-hemorrhagic inflammatory process, manifested by edema and haemorrhagic infiltration of the wall and also the presence of a bloody collection, intracavitary. Also, in the subserosal we can observe edema and hematomas of different sizes.



*Fig. 3 Haemorrhagic mesenteric lymph nodes*



*Fig. 4 Haemorrhagic-necrotic enteritis*

At the lung level we detected a pronounced edema, subpleural hemorrhages (fig. 5) and evident lobulation, consecutive to the dilatation of the interlobular spaces, in which a gelatinous or gelatin-hemorrhagic edema is present. A foamy, sometimes haemorrhagic fluid is leaking from the lung on section.

In the case of spleen, the anatomico-pathological lesions detected were also of a circulatory nature, presenting a much enlarged volume (2-4 times), dark red or purplish-blackish in colour (fig. 6), soft, friable consistency, with a large amount of blood leaking from the section. We also encountered numerous marginal splenic infarcts.



*Fig. 5 Subpleural haemorrhages*



*Fig. 6 Splenomegaly, dark-red, soft, friable consistency*

At the cardiac level, we also detected numerous haemorrhages in the form of suffusions or even haematomas (fig. 7).



**Fig. 7 Pericardial ecchymosis and petechiae**



**Fig. 8 Renal ecchymosis**

The kidneys show numerous haemorrhages on a congestive background and, sometimes, we can find them wrapped in a blood clot, as a result of haematomas forming retroperitoneally. Externally, the kidneys may have a “turkey egg” appearance due to punctiform haemorrhages (fig. 8). Haemorrhages are also present on the lining of the bladder.

### ***Conclusions***

1. African swine fever (ASF) is a highly contagious viral haemorrhagic disease of domestic and feral pigs caused by DNA virus of the *Asfarviridae* family
2. The diagnosis of certainty was made on the basis of the corroboration of the clinical signs and morpho-pathological lesions with laboratory tests
3. Symptomatology and morpho-pathological lesions detected in the animals studied classified the progressive form of the disease as acute
4. The laboratory tests used to identify ASFV were PCR and ELISA

### ***Acknowledgement***

This study is part of an undergraduate research carried out in the Faculty of Veterinary Medicine, *Spiru Haret* University, 2023.

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# ASPECTS OF AGGRESSIVE BEHAVIOR WITH GENETIC DETERMINATION IN THE BELGIAN MALINOIS BREED

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## ***Abstract***

*The past decade has seen rapid progress in the field of canine behavioral genetics. As dog aggression is considered a serious threat to public health there is a common perception that certain breeds are a greater risk of exhibiting aggressive behavior, which has contributed to the introduction of breed specific legislation. The culmination of half a century of behavioral testing and, more recently, genomic investigations was established by the identification of genes responsible for docility in silver foxes. In these way, after an experiment of more than 50 years, other studies has detected genes related to aggressive behavior that encode dopamine, serotonin and glutamate receptors in English Cocker, as well as the dopamine transporter gene was associated with impulsive behavior in Belgian Malinois, clearly demonstrating the genetic basis of behavior in canids.*

**Keywords:** *aggressive behavior, breed, Malinois, gene.*

## **Introduction**

Currently, specialized studies in the field of behavioral genetics use validated psychometric instruments such as the Canine Impulsivity Assessment Scale (DIAS), which scores elements of impulsivity, including a tendency for aggressive behavior, or the Positive and Negative Activation Scale (PANAS), which scores sensitivity to positive and negative stimulus (which can trigger aggressive response) [1, 2, 3].

Idiopathic aggression, that type of aggression for no reason, apparently without any stimulus, has been observed especially in the Belgian Malinois dog breed. But it is known that pathogenic mechanism of this type of unwanted behavior involves a dopaminergic gene that encodes the formation of the dopamine transporter (SLC6A3) and is relevant for aggression and other behavioral changes of the type of aggression attacks associated with the neurotransmitter dopamine. The dopamine transporter gene is involved in the production of the dopamine transporter, which is responsible for the presynaptic membrane in the neuron.

There is evidence to suggest that aggressive behavior in Belgian Malinois dogs breed can have a genetic component. Some aspects of aggression that may be genetically determined include:

- **reactivity** - that refers to the dogs tendency to over react to some stimuli, such as unknown dogs and people;
- **territoriality** - Belgian Malinois are known for their strong territorial instincts that can lead to aggressive behavior towards perceived intruders. This behavior can be more pronounced in dogs with a genetic predisposition;
- **prey Drive** - that refers to dogs instinct to pursue and capture prey. These behaviour is important for working dogs like Belgian Malinois but it can also lead to aggression towards smaller animals of people.
- **dominance** - dogs with a genetic predisposition to dominance may be more likely to exhibit aggressive behavior towards other dogs or even there owners. This behaviour can be exacerbated if the dog is not properly socialized or trained.

## **Material and methods**

Adverse behaviors as seizures, ‘glassy’ eyes and a tendency to bite the owner have been reported by many Belgian Malinois owners. These behavioral changes have been associated with certain genetic polymorphisms of the dopamine transporter gene (SCL6A3). For these reasons, in these paperwork we’ve proposed the genetic testing of 10 dogs belonging to the Belgian Malinois breed of different ages with different level of training, as well as their behavioral evaluation, in order to identify a predictive behavior associated with the specific gene polymorphism, with the aim of understanding some behavioral phenotypes related to SLC6A3 and expression of aggression in this breed.

### **Goals:**

- ✓ **No. 1:** genetic testing to determine predisposition to aggressive behavior;
- ✓ **No. 2:** comparative analysis of the tested genetic polymorphism (Poly A for the SCL6A3 gene) with the behavioral phenotypes of the study group and correlation of the results obtained from the questionnaire analysis with those obtained through genetic testing;
- ✓ **No. 3:** Formulation of working hypotheses for the Belgian Malinois training: ‘can training modify a genetic inherited behavior?’.

For testing the mutation of SLC6A3 gene, the laboratory is using TaqMan SNP test with peripheral venous blood. This genotyping method

uses the 5a nuclease activity of Taq polymerase, an enzyme used for in vitro DNA synthesis by the PCR technique, to generate a fluorescent signal during the polymerase chain reaction (PCR). For each single nucleotide polymorphism (SNP), the test uses two TaqMan probes that differ in sequence at SNP site, with one probe complementary to the wild-type allele and the other to the variant allele. The results were indicating one of the following genotype possibilities [3,4] (table 1):

**Table 1**

*The results of genetic testing for poly (A) in SLC6A3*

<b>Genotype</b>	<b>Interpretation</b>
<b>A0/ A0</b>	<b><i>IT IS NOT</i></b> associated with unwanted behavior
<b>A0/A10</b>	<b><i>IT IS NOT</i></b> associated with unwanted behavior
<b>A10/A10</b>	<b><i>IT IS NOT</i></b> associated with unwanted behavior
<b>A0/A22</b>	<b><i>MAY BE</i></b> associated with unwanted behavior
<b>A10/A22</b>	<b><i>MAY BE</i></b> associated with unwanted behavior
<b>A22/A22</b>	<b><i>IT IS</i></b> associated with unwanted behavior

## **Results and discussion**

It is important to note that while genetics can play a role in aggressive behavior, it is not the only factor. Environment, socialization and training also play a significant role in shaping a dog behavior. Responsible breeding practices and early socialization and training can help reduce the risk of aggressive behavior in Belgian Malinois dogs. The behavioral questionnaire contains a set of dog identification data, living environment data, medical and behavioral history. This questionnaire is inspired by the DBQ (dog behaviour questionnaire), the latter being one of the SAB test (Socially Acceptable Behaviour) questionnaires. This test contains several subtests and questionnaires, including DBQ.

By means of the behavioral questionnaire it is possible to see if there are associations between the Poly A(22) allele and changes in the behavior of the dogs within the batch, according to the owners report, if the aggressive behavior is due to a medical pathology, lack of movement or poor maintenance (Fig. 1). Also the questionnaire can be used reliably, in

correlation with the genetic test, and in determining the optimal sport training method for the Belgian Malinois.

Nume câine:	
Rasă:	Belgian Malinois
Vârsta:	9 ani
Sex:	M
Vârsta achiziției:	8 săptăm.
A mai avut și alți proprietari înainte?:	NU
Status hormonal:	Necunoscut
Vârsta primului estru (femele):	—
Ce tip de temperament are?	isteric
În ce mediu trăiește?:	apartment
Cât de des face activitate fizică?:	Nu mai face
De la ce vârstă s-a început dressajul?	9 săptăm.
De cât timp se lucrează cu câinele?	s-a lucrat puțin la team
Ce tip de dressaj?	Pușcă reală
Câte mese are pe zi?:	1
În ce constă hrana?:	Crackere
Suferă de vreo patologie?	DA <input checked="" type="radio"/> NU
Dacă da, ce diagnostic?	—
Este sub tratament?	DA <input checked="" type="radio"/> NU
Dacă da, ce anume?	—
Primește suplimente?	<input checked="" type="radio"/> DA NU
Dacă da, ce anume?	Senilife
A experimentat vreun episod de convulsii?	<input checked="" type="radio"/> DA NU
Ai observat la el/ea ochi lucioși, lipsa de răspuns la stimulii?	<input checked="" type="radio"/> DA NU
A avut episoade scurte și bruște de agresivitate fără factor declanșator, îndreptate către unul sau mai multe dintre următoarele persoane: proprietar, alte persoane sau alți câini	<input checked="" type="radio"/> DA NU
Are probleme de comportament?:	<input checked="" type="radio"/> DA NU
Dacă da, ce anume?:	Agresivitate excesivă
Prezintă semne de agresivitate față de persoane necunoscute?	<input checked="" type="radio"/> DA NU
Prezintă semne de agresivitate față de alți câini?	<input checked="" type="radio"/> DA NU
<p>+ foarte des decât alți câini nu sunt submisivi.</p> <p>+ este un câine introvertit.</p>	

**Fig.1 Behavioral questionnaire**

Based on a synergistic analysis from the behavioral genotypic and phenotypic point of view of the 10 cases studied we can conclude that specialized canine training (training) plays an extremely important role in managing unwanted behaviors and aggression, even in the presence of alleles of the mutant allele - Poly A (22) and that the genotype may not always correlate with the phenotype. Lack of training as well as mistakes in training can lead to the expression of the predisposition to aggression, just as correct and constant training leads to the control and lack of expression of unwanted behaviors.

## Conclusions

Behavioral genetics is a new field and there are still many unanswered questions. It has been shown that aggression varies by race and gender, and that behavioral changes are associated with genetic mutations. On the one hand, aggressive behaviors appear at an increased rate in certain breeds of dogs, this aspect being directly associated with genetic inheritance, and on the other hand, it has been shown that in the case of other breeds, heredity does not seem to be as important. Certain genes have been theorized to be partially responsible for aggressive behavior, but the extent to which they influence it remains to be determined.

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# DIFFERENCES IN VETERINARY CLINIC MANAGEMENT: ANALYZING CULTURAL, STRUCTURAL, AND OPERATIONAL VARIATIONS BETWEEN ITALY AND ROMANIA

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## ***Abstract***

*This article provides an in-depth analysis of the differences in veterinary clinic management between Italy and Romania. By examining the cultural, structural, and operational variations, we shed light on the unique approaches and challenges encountered in managing veterinary practices in these countries. The study explores the influence of cultural norms, regulatory frameworks, and operational factors on veterinary clinic management. The findings offer valuable insights for improving veterinary care and optimizing clinic operations in both Italy and Romania.*

**Keywords:** *Veterinary clinic management, cultural differences, structural variations, operational disparities, Italy, Romania*

## ***Introduction***

The effective management of veterinary clinics plays a vital role in providing quality care to animals and maintaining the overall well-being of veterinary practices. However, it is important to recognize that veterinary clinic management practices can vary significantly between countries. These variations may arise from cultural, structural, and operational factors inherent to each specific context. Understanding these differences is essential for identifying areas of improvement and implementing best practices that can optimize veterinary clinic management.

This article specifically focuses on analyzing and comparing the management strategies employed in veterinary clinics in Italy and Romania. Italy and Romania were chosen as case studies due to their distinct cultural, structural, and operational characteristics within the veterinary care domain. By examining these differences, the aim is to shed light on the specific factors that influence veterinary clinic management in each country and to identify potential areas for improvement and optimization.

The analysis in this study encompasses various dimensions of veterinary clinic management. Cultural factors, such as communication styles, patient

care approaches, and decision-making processes, are examined to understand how they shape the overall management strategies within veterinary practices in Italy and Romania. By exploring these cultural influences, the study aims to provide insights into how cultural norms impact the dynamics between veterinary professionals, clients, and staff members.

Additionally, the study delves into the structural variations in veterinary clinic management between Italy and Romania. This includes an examination of the regulatory frameworks that govern veterinary practices in each country, as well as an exploration of clinic size, ownership models, and specialized services. Understanding these structural differences is crucial for identifying the specific challenges and opportunities that exist within each country's veterinary care landscape.

Operational factors also play a significant role in veterinary clinic management. This study investigates operational disparities such as resource availability, technology adoption, and continuing education and professional development opportunities. By analyzing these factors, the aim is to gain insights into how they impact the overall efficiency, effectiveness, and quality of veterinary clinic management in Italy and Romania.

The findings of this study are expected to provide valuable insights and recommendations for improving veterinary clinic management in both Italy and Romania. By recognizing the cultural, structural, and operational factors that contribute to the differences observed in veterinary clinic management practices, veterinary professionals can adapt their strategies, implement best practices, and overcome challenges specific to each country's context. Ultimately, the goal is to enhance the overall management and delivery of veterinary care in both Italy and Romania, leading to improved animal welfare and client satisfaction.

It is important to note that while this study focuses on the veterinary clinic management practices in Italy and Romania, the insights and recommendations generated can also serve as a basis for comparative analyses in other countries and regions. By expanding the scope of research in this field, a more comprehensive understanding of veterinary clinic management practices can be achieved, benefiting veterinary professionals and animals worldwide.

### ***Materials and Methods***

To investigate the differences in veterinary clinic management between Italy and Romania, a multi-faceted approach was employed in this study.

The following methods were utilized to collect comprehensive and reliable data:

1. Literature Review: A thorough literature review was conducted to gather existing research and knowledge on veterinary clinic management in both Italy and Romania. Relevant academic journals, books, industry reports, and online resources were consulted to establish a foundation of understanding [1, 2, 3, 4, 5].
2. Surveys: Surveys were designed and administered to veterinary professionals, clinic owners, and clients in Italy and Romania. The surveys aimed to gather firsthand information on various aspects of veterinary clinic management, including cultural influences, structural variations, and operational disparities. The survey questions were carefully crafted to address specific research objectives and provide quantitative data for analysis.
3. Interviews: Semi-structured interviews were conducted with a selected sample of veterinary professionals, clinic owners, and clients. These interviews allowed for in-depth exploration of their experiences, perspectives, and insights regarding veterinary clinic management in their respective countries. The interviews provided qualitative data, offering valuable subjective information and personal anecdotes related to cultural, structural, and operational aspects.
4. Site Visits: Site visits were carried out at a representative sample of veterinary clinics in Italy and Romania. These visits involved direct observation and analysis of clinic practices, infrastructure, and operations. The researchers examined the physical layout of the clinics, the availability and usage of technology and equipment, the range of services offered, and the overall efficiency of clinic management. The site visits provided firsthand insights into the practical implementation of veterinary clinic management strategies and allowed for comparisons between the two countries.

By combining these research methods, a comprehensive understanding of the cultural, structural, and operational factors influencing veterinary clinic management in Italy and Romania was obtained. The data collected through literature review, surveys, interviews, and site visits were analyzed to identify patterns, trends, and significant differences between the two countries.

It is important to note that ethical considerations, such as obtaining informed consent from participants and maintaining confidentiality, were adhered to throughout the data collection process. The research was

conducted with integrity and in accordance with ethical guidelines to ensure the reliability and validity of the findings.

## ***Results and Discussion***

### **Cultural Influences on Veterinary Clinic Management**

**Cultural Context in Italy:** Italy's rich cultural heritage significantly influences veterinary clinic management in the country. The emphasis on tradition and personal relationships shapes the approach to client communication, patient care, and decision-making processes within veterinary practices. Italian veterinary clinics often prioritize building strong personal connections with clients, fostering trust and loyalty over time. The cultural values of hospitality, warmth, and interpersonal relationships are deeply embedded in the management strategies of Italian clinics. Veterinarians in Italy strive to create a welcoming and familiar environment for clients, where they feel valued and understood. This cultural context also influences the communication style, with veterinarians often adopting a compassionate and empathetic approach when interacting with clients.

**Cultural Context in Romania:** Romania has its unique cultural aspects that impact veterinary clinic management. The respect for authority and collective decision-making influence the dynamics between veterinary professionals, clients, and staff members. In Romanian veterinary clinics, there is a strong sense of community, where clients often rely on word-of-mouth recommendations and community networks to choose a veterinary practice. Building trust and establishing a reputation within the community are vital for the success of veterinary clinics in Romania. Veterinarians in Romania often collaborate with other professionals, such as breeders and local authorities, to ensure the well-being of animals in the community. The cultural values of respect, trust, and collaboration shape the management strategies in Romanian clinics, fostering a sense of unity and cooperation among veterinary professionals and clients.

**Table 1: Comparison of Veterinary Clinic Management Factors in Italy and Romania**

<b>Management Factors</b>	<b>Italy</b>	<b>Romania</b>
Communication	Emphasis on building personal relationships with clients	Strong sense of community, reliance on word-of-mouth recommendations
Patient Care	Personalized approach to care	Emphasis on collective decision-making
Decision-making	Collaborative decision-making process	Respect for authority, collective decision-making
Regulations	Regulated by Ordine dei Medici Veterinari	Regulated by Colegiul Medicilor Veterinari
Clinic Size and Infrastructure	Smaller patient capacities, personalized approach to care	Larger capacities, accommodate higher volume of patients
Resource Availability	Greater access to advanced diagnostic equipment, wider range of medications	Varied resource availability based on proximity to manufacturing hubs
Technology Adoption	Advanced adoption of digital platforms for communication and record-keeping	Transitioning to more technology-driven management systems
Education and Professional Development	Access to a wide range of continuing education opportunities	Availability of specialized training programs and conferences

### **Structural Variations in Veterinary Clinic Management**

**Regulatory Frameworks:** Italy and Romania have distinct regulatory frameworks governing veterinary practices. In Italy, veterinary practices are regulated by the Ordine dei Medici Veterinari, while in Romania, the regulatory body is the Colegiul Medicilor Veterinari. These regulatory frameworks encompass licensing procedures, practice ownership models, professional associations, and legal requirements. Understanding the regulatory landscape is essential for complying with industry standards and optimizing veterinary clinic management. The regulatory frameworks may influence the ownership structure of clinics, the scope of services offered, and the professional responsibilities of veterinarians. It is crucial for veterinary clinics to navigate and adhere to these regulations to ensure the delivery of quality care and efficient management.

**Clinic Size and Infrastructure:** The size and infrastructure of veterinary clinics vary in Italy and Romania. Italian clinics often have smaller patient capacities, emphasizing a more personalized approach to care. These clinics

may focus on providing specialized services and developing strong relationships with a limited number of clients. In contrast, Romanian clinics may have larger capacities, accommodating a higher volume of patients. The larger size allows for a broader client base and a wider range of services. The clinic infrastructure, including facilities, equipment, and specialized services, also varies between the two countries. For instance, Italian clinics may have a greater focus on specialty services like dermatology or ophthalmology, while Romanian clinics might offer a broader range of services to cater to diverse patient needs. The differences in clinic size and infrastructure impact resource allocation, patient flow management, and the overall patient experience.

### **Operational Disparities in Veterinary Clinic Management**

**Resource Availability:** The availability and accessibility of resources, including veterinary supplies, medications, and diagnostic tools, play a crucial role in clinic management. Variations in resource availability influence the quality of patient care, clinic efficiency, and the ability to invest in technological advancements. For instance, Italian clinics may have greater access to advanced diagnostic equipment and a wider range of medications due to their proximity to manufacturing hubs. On the other hand, Romanian clinics may face challenges in resource availability, especially in rural areas or regions with limited access to veterinary suppliers. Resource management and procurement strategies differ between the two countries, affecting the overall operational efficiency and the ability to deliver optimal care to patients.

**Technology Adoption:** The adoption and integration of technology within veterinary clinics vary across countries. Factors such as access to veterinary software systems, electronic medical records, telemedicine, and online appointment scheduling impact clinic operations and client satisfaction. Italian clinics may have a higher adoption rate of technology, utilizing digital platforms for record-keeping, communication, and client engagement. Romanian clinics, particularly those in remote or underserved areas, may face challenges in technology adoption due to infrastructure limitations or financial constraints. The integration of technology in veterinary clinic management can streamline processes, enhance communication, and improve efficiency, ultimately benefiting both veterinary professionals and clients. Disparities in technology adoption between Italy and Romania may contribute to variations in clinic management practices and service delivery.

**Continuing Education and Professional Development:** The commitment to continuing education and professional development differs between Italy and Romania. The availability of specialized training programs, conferences, and workshops influences the knowledge and skillset of veterinary professionals, ultimately impacting clinic management practices. In Italy, veterinarians have access to a wide range of continuing education opportunities, including conferences, seminars, and certifications in specialized fields. These opportunities contribute to ongoing professional development and the adoption of advanced techniques and treatments. In Romania, while continuing education options are available, they may be relatively limited in comparison. The discrepancies in continuing education resources and opportunities may influence the level of expertise, innovative practices, and up-to-date knowledge within veterinary clinics. Continuous learning and professional development are vital for enhancing clinic management strategies and ensuring the delivery of high-quality veterinary care.

By examining these cultural, structural, and operational factors, this study sheds light on the diverse approaches to veterinary clinic management in Italy and Romania. The findings contribute to a deeper understanding of the contextual influences and provide valuable insights for improving clinic management practices in both countries.

### ***Conclusions***

The findings of this study highlight the significant impact of cultural influences on veterinary clinic management in Italy and Romania. Understanding the cultural context is crucial for effective communication, client satisfaction, and building trust within the veterinary practice. In Italy, the emphasis on tradition and personal relationships guides the approach to client care, while in Romania, the respect for authority and community dynamics shape the management strategies. Recognizing and respecting these cultural nuances can lead to improved client relationships, better adherence to treatment plans, and increased client loyalty.

Moreover, the study identified structural variations in veterinary clinic management between the two countries. The regulatory frameworks governing veterinary practices differ in Italy and Romania, necessitating an understanding of the legal requirements and compliance standards specific to each country. Clinic size and infrastructure also play a role in shaping management practices. Italian clinics, often characterized by smaller patient capacities, focus on personalized care and specialized services, while Romanian clinics may have larger capacities to accommodate a higher

volume of patients and offer a broader range of services. These structural differences impact resource allocation, patient flow management, and the overall patient experience.

Operational disparities were also identified, particularly in terms of resource availability, technology adoption, and continuing education and professional development. Resource availability, including veterinary supplies, medications, and diagnostic tools, can affect the quality of care and overall clinic efficiency. Technology adoption varies, with Italian clinics generally being more technologically advanced, while Romanian clinics may face limitations in accessing and integrating digital solutions. Furthermore, differences in continuing education and professional development opportunities influence the knowledge and skills of veterinary professionals, which, in turn, impact clinic management practices.

In conclusion, this study highlights the importance of considering cultural, structural, and operational factors in veterinary clinic management in Italy and Romania. Recognizing and understanding these variations can help veterinary professionals make informed decisions, implement best practices, and optimize their clinic management strategies. By fostering knowledge exchange, collaboration, and cross-cultural understanding, we can contribute to the advancement of veterinary clinic management practices in both countries, ultimately improving the quality of care provided to animals and strengthening the veterinary profession as a whole.

It is important to note that while this study focused specifically on Italy and Romania, the findings and recommendations can serve as a basis for comparative studies and insights into veterinary clinic management practices in other cultural and regional contexts. Further research and exploration of these factors can contribute to a more comprehensive understanding of veterinary clinic management globally.

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