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This thesis contains:

Summaries (Romanian, English, French)
Extended general part 55 pages;
Extended own research part 137 pages;
Tables: 11;
Figures full color: 111;
References: 303 references.

SUMMARY

Of the PhD thesis entitled

RESEARCH ON THE EPIDEMIOLOGY, DIAGNOSIS AND CONTROL OF CANINE BABESIOSIS IN WESTERN ROMANIA

The paper is structured in two parts the first part: ***Bibliographical study*** - and second part: ***Own researches.***

The first part contains four chapters:
CAP. 1 Etiology of canine babesiosis
CAP. 2 Epidemiological aspects in canine babesiosis
CAP. 3 Diagnosis of canine babesiosis
CAP. 4 Control of canine babesiosis

In **the first chapter** contains updated information available in the literature on the etiology of canine babesiosis. In the foreground is the disease history and taxonomic classification followed by a review of the species included in the genus *Babesia*. Also, here are presented information about intermediate forms of development and life cycle of the parasite, in two chapters that include: development in the vertebrate host and in the invertebrate host.

In the **second chapter** epidemiological aspects of possible hosts, geographical distribution of the infection, disease transmission pathways and the factors favoring receptivity, are addressed.

Chapter three describes the diagnostic techniques used in canine babesiosis including diagnosis on epidemiological data, clinical pathology and laboratory findings. The laboratory diagnosis subchapter presents issues about highlighting piroplasms using colored smear method, Percoll gradient method, immunofluorescence method and polymerase chain reaction technique.

In **chapter four** references are made in the control of canine babesiosis. General preventive measures are presented including prophylactic administration of acaricides on

animal, control of ticks in the environment, vaccination and chemoprophylaxis. Specific therapy of the disease, with babesicid terapy and supportive therapy are also described here.

The second part contains seven experimental chapters which make reference to the information described in the first part:

Chap. 5. Questionnaire-based epidemiological investigation of babesiosis and other blood pathogens in dogs from western Romania
Chap. 6. Seroprevalence studies based on immunoassay techniques regarding the spreading of canine babesiosis in western Romania
Chap. 7. Prevalence of tick species, in dogs, in western Romania
Chap. 8. Comparative study of tick species found in dogs, using usual methods and Scan Electrono Microscopy (SEM)
Chap. 9. Molecular survey of canine babesiosis
Chap. 10. Clinical observations, hematological changes and therapy in acute canine babesiosis caused by *Babesia canis*
Chap. 11. General conclusions

Chapter 5 presents the results of a questionnaire-based epidemiological survey of the evolution of canine babesiosis and other blood pathogens in dogs in western Romania. The study was conducted as a starting point for further investigations, in order to obtain a more complex and real picture of the spreading of the main blood pathogens in the canine population of western Romania (Arad, Bihor, Caras-Severin and Timis Counties) from February 2010 to February 2011. The investigation addressed a questionnaire of 27 questions for veterinary practitioners (n = 47) of private clinics in large urban (Arad, Oradea, Resita, Timisoara and Caransebes) and rural areas.

Study results showed that:

- Out of 47 veterinary units, only 12 (25.5%) practiced laboratory tests for the diagnosis of blood pathogens, on a regular basis;

- The most commonly used diagnostic techniques were colored blood smear, followed by direct examination of blood drop between slides and rapid diagnostic tests;

- Heartworm disease was reported in 14 (29.8%) out of the 47 veterinary units investigated;

- Out of 47 investigated veterinary units in 18 (38.3%) cases of babesiosis have been reported, with seasonal occurrence and the most common clinical signs observed were: fever, hemoglobinuria and icterus;

-All diagnosed cases of babesiosis were indigenous infection;

-Cases of ehrlichiosis were diagnosed in one unit in Bihor and Arad Counties;

- So far, veterinarians from the western part of the country - Arad, Bihor, Caras-Severin and Timis Counties – didn't diagnosed cases of anaplasmosis and hepatozoonosis.

Chapter 6 illustrates the results of seroprevalence studies on the spread of canine babesiosis in the west of Romania using enzyme immunoassay techniques. This chapter is the skeleton of the thesis and is composed of three subchapters:

- Seroprevalence of *Babesia canis* infection in clinically healthy dogs from Banat region;

- Serological survey of *Babesia gibsoni* infection in dogs from western Romania;
- Seroepidemiological screening of *Babesia canis* in association with other tick – borne pathogens of dogs from western Romania;

❖ The first subchapter provides information about the seroprevalence of *B. canis* infection in clinically healthy dogs in Banat area. In this study, conducted from August 2010 to May 2011, 197 dogs asymptomatic (clinically healthy) with owner, randomly selected from 25 settlements in Banat region, were investigated. From each animal a blood sample was collected and the serum obtained was processed for evidence of *B. canis* antibodies by indirect immunofluorescence technique using MegaScreen FLUOBABESIA canis® kit (Diagnostik Megace, Hörbranz, GmbH, Austria).

From each animal in the study epidemiological data regarding age, gender, breed, habitat and lifestyle were obtained from the owners. They declared that all the animals were born in the study area and never have been abroad.

Of the 197 clinically healthy dogs investigated in the study, 39 (19.8%) were seropositive for *B. canis*. Statistical analysis showed significant differences ($p < 0.05$) in the distribution of infection among dogs in rural (28.4%, 19/67) and urban areas (15.4%, 20/130). The seroprevalence of canine babesiosis was significantly higher in hunting dogs (15/34, 44.1%) compared with pet dogs (8/65, 12.3%, $p = 0.0006$) and guard dogs (16/98, 16.3%, $p = 0001$). Using of dogs for hunting was the only factor (OR = 4.57, 95% CI = 2.1-10.2, $p = 0.002$) positively associated with seroprevalence in dogs investigated and can be considered a risk factor for acquiring the infection.

No significant differences were found between pet dogs and guard dogs. No associations were recorded between seroprevalence and age groups of infected animals. Similarly, the number of seropositive male dogs (22/105; 20.9%) and female (17/92, 18.5%), and the number of seropositive pure breeds (24/116, 20.6%) and mixed breeds (15/81, 18.5%) did not differ significantly ($p = 0.673$ $p = 0.457$ respectively) between them.

Investigation confirms indirectly the evolution of canine babesiosis caused by *B. canis* in the surveyed area.

❖ Subchapter two includes data on the results of serological surveillance of *Babesia gibsoni* infection in dogs in the western part of Romania. The study was conducted from March 2011 to April 2013, on a total of 308 dogs aged between two months and 13 years. Carnivores in the study were randomly selected from the following categories: owned dogs, stray dogs living with other dogs in shelters and street dogs (stray dogs) with unknown epidemiological situation. Dogs in the study lived in five western counties (Arad, Bihor, Caras - Severin, Satu - Mare and Timis) of the country. After blood collection, serum obtained from each animal was examined by indirect immunofluorescence technique to emphasize the antigen-antibody complex using MegaScreen FLUOBABESIAGIBSONI ® kit (Diagnostik MegaCor, Hörbranz, GmbH, Austria) following the manufacturer's recommendations. Age, gender, breed, origin (rural or urban) and lifestyle (dogs with owner, stray dogs and dogs from dog shelters) of dogs were considered.

Seroepidemiological screening conducted in five counties in western Romania (Arad, Bihor, Caras-Severin, Satu-Mare and Timis) by indirect immunofluorescence technique on 308 dogs showed seropositivity for the infection with *B. gibsoni* in 2.9% (11/308) of the dogs.

There were not found positive associations between seroprevalence of infection and the epidemiological data including: origin, age, breed, gender, habitat and lifestyle of dogs.

❖ In the third chapter information on seroepidemiologic screening results on *Babesia canis* parasitism in association with other pathogens transmitted by ticks in dogs in western Romania, are presented. The research was conducted between June 2011 - May 2013, a total of 221 dogs with suggestive clinical signs for tick-borne diseases. The enrolled carnivores in the study were provided from 25 localities of Arad (n = 38), Bihor (n = 34), Caras - Severin (n = 78) and Timis (n = 71) counties. Dogs were randomly chosen as part of the following categories: owned dogs and stray dogs with unknown epidemiological situation. From each animal in the study blood was collected, and after plasma expressing indirect immunofluorescence diagnostic method was used for serological screening of *B. canis, A. phagocytophilum, B. burgdorferi* sensu lato (s.l.) and *E. canis* antibodies. In order to achieve the intended purpose the kit MegaScreen® FLUOVET® (Diagnostik Megacor, GmbH, Austria) was used.

A seroprevalence of 15.8% was found for *B. canis*, 16.3% for *E. canis*, 5.5% for *A. phagocitophylum* and 25.9% for *B. burgdorferi* s.l. The following associations were found: *B. canis* + *E. canis* 1.8%, *B. canis* + *B. burgdorferi* s.l. 3.2%, *B. canis* + *A. phagocitophylum* 1.8%, *B. canis* + *E. canis* + *A. phagocitophylum* 0.45%, *B. canis* + *E. canis* + *B. burgdorferi* s.l. 0.45% and *B. canis* + *A. phagocitophylum* + *B. burgdorferi* s.l. 0.9%. In each locality, at least one pathogen form the four tested was found.

The results confirm the important role of dogs as reservoir in the epidemiology of tick-borne diseases in Romania and highlight that the presence of antibodies against the four pathogens tested is an indirect evidence of the evolution of babesiosis, ehrlichiosis, anaplasmosis and borreliosis in various forms (occult, symptomatic) in the surveyed area.

The seventh chapter provides information about the prevalence of tick species in dogs in the western part of Romania, based on morphological characters observed by classical methods, as a starting point in assessing the risk of transmission of infectious agents and parasites. In this regard, 793 ticks were collected from 113 dogs infected naturally from Timis, Arad, Caras-Severin, Bihor and Satu Mare counties, which were presented to veterinary clinics in the studied area or to veterinary clinics of Faculty of Veterinary Medicine Timisoara, between April 2011 and May 2013. In the anamnesis origin (rural or urban), habitat (yard, apartment, etc.), the body regions of tick attachment, and any previous treatments to the sampling of ticks, was noted. Arthropods were removed from the animal skin with a forceps, collected in containers with added 70% ethanol and transported to the laboratory for identification under optical stereomicroscope.

Overall, the prevalence of tick species investigated in the canine population of the surveyed area was: *Ixodes ricinus* (57.7%), *Rhipicephalus sanguineus* (32.7%), *Dermacentor reticulatus* (6.03%), *Dermacentor marginatus* (0.8 %) and *Haemaphisalis punctata* (0.8%). The risk of transmission of *Babesia* parasites in dogs in the surveyed area was confirmed by the existence of principal vector namely *Dermacentor reticulatus* and other vector tick species, *Rhipicephalus sanguineus*. From all dogs sampled 85% were infested with a single species of ticks, while 15% was found to be co-infested of which *I. ricinus* and *Rh. sanguineus* association was the most frequent. Prevalence of tick parasitism was significantly higher ($p<0.05$) in untreated animals group than in the treated animals. The most frequent

localization of ticks on dogs was the head region, followed by the limbs and neck. *I. ricinus* was found as the most prevalent tick species and may be a risk factor for canine investigated population, since it is a major reservoir and vector for the zoonotic pathogen *Borrelia burgdorferi*. The possibility of transmission of pathogens such as *Babesia* spp, *Ehrlichia* spp and *Anaplasma* spp in dogs in the study was highlighted by the presence of the tick *Rh. sanguineus*.

Chapter eight presents the results of a comparative diagnostic study of species of parasitic ticks in dogs by usual methods and scanning electron microscopy (SEM). Observations were made on ticks collected from naturally infested dogs with Ixodids from Timis, Arad and Bihor counties. In the first phase, each tick was identified in optical stereomicroscope and morphological characters were identified and confirmed by scanning electron microscope (Quanta 250 SEM).

Study results showed that species of ticks in dogs can be identified at the genus and species level by optical microscopy and scanning electron microscopy (SEM) or, for greater certainty, by summing data from both methods. Optical microscope has the advantage of presenting morphological characters in a wider color range, allowing color highlighting of differences between different types of ticks. At the species level, a precise identification in the optical microscope images can be obtained corroborating several plans of view. Also scanning electron microscopy technique (SEM) has proven to be a useful tool in characterizing species of ticks in dogs, as morphological elements can be observed with greater precision and in detail and used as basic criteria in ticks identification. In addition, some small morphological structures such as shape and size of setae, porous areas, festons and genital and anal pores can be observed in detail only the scanning electron microscope.

Chapter nine entitled "Molecular biology studies in canine babesiosis" provides information about *Babesia* species found in dogs in western Romania determined by molecular biology techniques and complete the etiology of canine babesiosis with new data.

The study was conducted from October 2009 to May 2013 on a total of 66 dogs from two western (Arad and Timisoara) and three northwestern counties (Bihor, Satu - Mare and Salaj) of Romania. From each animal blood samples were taken. The dogs investigated had two or more clinical signs suggestive of canine babesiosis such as fever, icterus and hemoglobinuria. During the clinical examination data have been obtained from the owners of animals such as origin, history of travel to other areas including neighboring countries, age, breed, gender or previous contact with a tick vector. As a first step, on the day of blood collection, individual blood smears were made from the biological material, subsequently Diff - Quick® stained and examined under the optical microscope in order to reveal possible piroplasms in red blood cells. Molecular analysis and determination of the species included polymerase chain reaction technique, restriction fragment length polimorphism analysis using enzyme digestion H*in*f and T*aq*I, targeting 18S SSU-rRNA gene. In order to confirm PCR - RFLP method, all the isolates of *B. gibsoni* and 17 isolated from *B. canis* randomly selected were sequenced.

On microscopic examination (Ob x 100) of Diff - Quick® stained blood smears in 52 cases (78.8%) the presence of two pear shape (teardrop) sectioned forms, with the size of half of the erythrocyte (2.5 - 5.0 μm), and morphologically consistent with large babesia species s was observed in the red blood cells. In 10 (15.2%) samples the presence of intense colored

hull forms, reduced in size, with an oval appearance was reported. Also, in four (6.1%) samples the presence of a parasitic element has been not observed. In all samples processed molecular analysis of 18S rRNA gene fragment using primers Piro Piro A and B showed the presence of bright bands at ~ 410 base pairs. The size of the amplification products suggested that the organisms genetically characterized belong to the genus *Babesia*. Further analysis of restriction fragment length polymorphism (RFLP) of the PCR product with H*inf* and T*aq*I enzyme showed parasitism with *B. canis* (52 cases, 78.8%) and *B. gibsoni* (14 cases, 21.2%). In all cases, the results were confirmed by sequencing.

The territorial distribution of infection by county was: *B. canis* in Arad (21, 40.4%), Bihar (3, 5.8%), Satu - Mare (12, 23.1%) and Timiş (16, 30.1%) and *B. gibsoni* in Satu - Mare (12, 85.7 %) and Salaj (2, 14.3%), respectively. No positive associations were found ($p > 0.05$) between species distribution and epidemiological factors investigated, including age and gender of dogs. Infections with *B. gibsoni* were positively associated ($p < 0.001$) with fighting dogs breeds (12 American Pit Bull Terriers and American Staffordshire Terriers). Positive association with the occurrence of the species *B. gibsoni* in fighting dogs, without knowing any previous contact with ticks, suggests the existence of factors independent of arthropod vector such as the transmission of infection from dog to dog by bite or vertical transmission.

Percentage of *B. canis* infected dogs reported by owners with previous contact with ticks was significantly higher ($p < 0.001$) than dogs who have not reported any previous contact with arthropod vector (82.7% vs. 17.3 %).

The importance of this study lies in the fact that is the first reference to molecular identification of *B. canis* in the western and north-western Romania. Also, this report contains the first record of the specie *B. gibsoni* in Romania confirmed by sequencing, providing important information at national and continental level about the appearance of this species as pathogen in canine population.

Chapter ten illustrates the results of clinical observations, hematology and blood chemistry investigations, and also therapeutic approach in acute canine babesiosis caused by *B. canis*. Therefore, in this chapter are described and evaluated in a broader context the most important problems that arise in acute infections in dogs naturally infected with *B. canis* species. In addition, the specific therapeutic approach and support healing is provided.

The study was conducted on a total of 20 dogs, with owner, from October 2009 to May 2013. Routine physical examination of dogs revealed several clinical signs suggestive of canine babesiosis such as anorexia, anemia of mucous membranes, icterus, fever, and hemoglobinuria. Therefore, the general clinical examination focused primarily on quantifying these five clinical signs in all animals studied.

In a first step, for all dogs blood samples were collected and stained smears Diff - Quick® (Medion Diagnostics GmbH, Düdingen, Switzerland) were made. The presence of protozoa in the blood smear was observed by microscopic examination in all dogs investigated and subsequently identification of the species *B. canis* was performed by PCR-RFLP. Further, at the request of specialist veterinarian and owners a number of haematological and biochemical tests have been made. For each parameter considered a number of dogs was investigated. Complete blood counts were performed using automated hematology analyzer Cell-Dyn 3700 (Abbot) and serum biochemical analyzes were performed using multiparameter analyzer EOS BRAVO FORTE (Hospitex Diagnostics®,

Italy). Determinations were done for the following parameters: hematocrit (total number of dogs evaluated for the parameter n = 20), hemoglobin (n = 11), the number of red blood cells (n = 13), mean erythrocytes volume (n = 9), red blood cell hemoglobin mean (n = 9), mean red blood cell hemoglobin concentration (n = 9), the coefficient of variation of the size of red blood cells (n = 9), platelets (n = 9), number of white blood cells (n = 12) and the percentage of neutrophils (n = 16), lymphocytes (n = 16), monocytes (n = 16), eosinophils (n = 16) and basophils (n = 16). Quantification of the biochemical parameters was based on the determination of creatinine (n = 12), serum albumin (n = 9), alkaline phosphatase (n = 12), bilirubin (n = 7), gamma - glutamyl transferase (n = 10), alanine aminotransferase (n = 11), aspartate amino-transferase (n = 8) and total protein (n = 10).

Finally, each dog with acute babesiosis received specific and symptomatic treatment. In 18 dogs specific treatment consisted in a single subcutaneous administration of Imidocarb dipropionate (Imizol®, Schering-Plough Animal Health) at a dose of 2.4 mg/kg (0.25 ml/10 kg bw) with booster administration, in two weeks. Two dogs were treated with doxycycline hyclate (RONAXAN®, Merial) at a dose of 10 mg/kg for two weeks, with a single daily administration. After each administration the presence or absence of side effects was evaluated. In each treated animal, in addition to the specific therapy, restoring the fluid and electrolyte imbalance was aimed. This was achieved by administration of poliionic solutions such as physiological saline or Ringer's serum, Ringer - lactate solution, Duphalyte as well as 5% or 10% glucose.

Study results showed that the frequency of clinical signs, evaluated at 20 dogs investigated and diagnosed with acute babesiosis caused by *B. canis* was 95% for anorexia, 90% for fever and hemoglobinuria, 70% for the presence of anemic mucosal and 15% for icterus.

Haematological profile analysis showed the presence of significant and characteristic modifications including eritrocitopenia, decreased hematocrit, thrombocytopenia of varying degrees (severe, moderate) and monocytosis.

Biochemical profile analysis demonstrated that significant and characteristic modifications caused by *B. canis* such as increased bilirubin and aspartate aminotransferase activity.

Specific medication consists of a single administration of Imidocarb propionate (Imizol®, Schering-Plough Animal Health) at a dose of 2.4 mg/kg (0,25 ml/10 kg bw) and the second booster administration at two weeks in all cases, resulted in significant improvement of health status in a relatively short time (24-48 h) but after administration, in addition to pain, parasimpaticomimetic adverse reactions occur.

In the case of doxycycline hyclat administration (RONAXAN ®, Merial), at a dose of 10 mg/kg for two weeks with a single administration every day, no side effects were observed and the drug was very well tolerated. But the health recovery measured by the disappearance of the characteristic clinical signs of babesiosis, is late.

In **chapter 11** are presented the 27 general conclusions that emerged from this thesis. These are:

- Epidemiological survey conducted in 47 veterinary units from western Romania showed that only 25.5% of them practice laboratory tests for the diagnosis of blood

pathogens. This indicates a poor knowledge and/or negligence of veterinarians in the theme.

- The most commonly used diagnostic techniques, by practicing veterinarians, in veterinary units surveyed in Western Romania are colored blood smear, followed by direct examination of blood drop between slides and use of diagnostic quick tests.

- Frequency of diseases investigated in veterinary units considered, in descending order, was: 38.3% for babesiosis, 29.8% for dirofilariosis and 4.3% for ehrlichiosis. Anaplasmosis and hepatozoonosis were not diagnosed by the veterinarians in the investigated units.

- Veterinarians from the investigated units reported that the most common clinical signs of babesiosis were fever, hemoglobinuria and inconstant icterus.

- *B. canis* infection seroprevalence determined by indirect immunofluorescence in 197 asymptomatic dogs Banat region was 19.8%. During the investigation the percentage seropositive dogs from rural areas was significantly higher compared with dogs from urban areas and seroprevalence of *B. canis* infection in hunting dogs was significantly higher compared to dogs with a different lifestyle. No significant differences were found between pet dogs and guard dogs lifestyle and seroprevalence as well as between ages or genders of infected animals.

- The use of dogs in hunting can be considered a risk factor for acquiring infection with *B. canis*.

- Seroprevalence of infection with *B. gibsoni* in five counties in western Romania (Arad, Bihor, Caras-Severin, Satu-Mare and Timis), for a number of 308 dogs monitored by indirect immunofluorescence technique, was 2.9%, without any association between prevalence and origin, age, breed, gender, habitat or lifestyle of dogs.

- Seroprevalence by indirect immunofluorescence technique, performed in 221 dogs suspected of tick-borne diseases in 25 localities in Arad, Bihor, Caras - Severin and Timis revealed a prevalence of 15, 8% for *B. canis*, 16.3% for *E. canis*, 5.5% for *A. phagocitophylum* and 25.9% for *B. burgdorferi s.l.* in each locality identifying at least one of the tested pathogens.

- The most frequent associations between pathogens antibodies were: *B. canis* + *B. burgdorferi* s.l. 3.2%, *B. canis* + *E. canis* 1.8%, *B. canis* + *A. phagocitophylum* 1.8%, *B. canis* + *A. phagocitophylum* + *B. burgdorferi* s.l. 0.9%, *B. canis* + *E. canis* + *A. phagocitophylum* 0.45% and *B. burgdorferi* s.l. 0.45%.

- The presence of antibodies to *B. canis, E. canis*, *A. phagocitophylum* and *B. burgdorferi* s.l. in dogs suspected of tick-borne diseases in the western part of Romania confirms their role as important reservoir in the epidemiology of tick-borne diseases with zoonotic character of the surveyed area. Also, the data obtained show the geographic expansion of emerging diseases transmitted by ticks in areas previously considered "free" of our country and generally in Europe.

- Survey of tick ectoparasitism in 113 dogs in three western counties (Arad, Bihor and Timis) has shown a prevalence of 57.7% for *Ixodes ricinus*, 32.7% for *Rhipicephalus sanguineus*, 6.03% *Dermacentor reticulatus* and for 0.8% *Dermacentor marginatus* and *Haemaphisalis punctata,* respectively

- 85% of the dogs were infested with a single species of ticks, while 15% was found to be co-infested. The most frequent co-infestation was *I. ricinus* and *Rh. sanguineus.* A significantly higher prevalence was found in the untreated animals than in the animals treated for ectoparasites. The most common sites of attachment of ticks on dogs were the head, followed by the limbs and neck.

- Existence of tick species *Dermacentor reticulatus* and *Rhipicephalus sanguineus* revealed the risk of transmission of *Babesia* parasites in dogs in the western Romania.

- *I. ricinus* species dominance and frequent occurrence of *Rh. sanguineus* in Arad, Bihor and Timis counties highlight the possibility of transmitting zoonotic pathogens such as *Borrelia burgdorferi sl, E. canis, Anaplasma spp* and *Babesia spp.*

- Optical microscopy study of major morphological characters in ticks revealed the possibility of examination of ticks in a wide color gamut, allowing highlighting of color differences between different genres and identification to species level.

- Scanning electron microscopy technique (SEM) has proven to be a useful tool in characterizing species of parasitic ticks in dogs, with greater precision and in detail for the morphological elements used as basic criteria to differentiate ticks species. Thus, some small morphological structures such as shape and size of porous areas, the festons, and genital and anal pores can be observed in detail only by the scanning electron microscope.

- Molecular biology investigations performed in 66 symptomatic dogs with canine babesiosis in two western counties (Arad and Timis) and three north - western counties of Romania (Bihor, Satu - Mare and Salaj) showed a parasitism with species *B. canis* (52 cases, 78.8%) and *B. gibsoni* (14 cases, 21.2%).

- Territorial distribution of infections in symptomatic dogs, by counties, was as follows *B. canis* in Arad (21, 40.4%), Bihar (3, 5.8%), Satu - Mare (12, 23.1%) and Timis (16, 30.1) and *B. gibsoni* in Satu - Mare (12, 85.7%) and Salaj (2, 14.3%), respectively.

- No positive associations were found ($p > 0.05$) between *Babesia* species distribution and epidemiological factors investigated, including age and gender of dogs. In contrast, infections with *B. gibsoni* was positively associated ($p < 0001$) with fighting dogs breed (12 American Pit Bull Terriers and American Staffordshire Terrier).

- Positive association of the occurrence of *B. gibsoni* in fighting dog breeds, without any known previous contact with ticks, suggests the existence of factors in the acquiring infection with this species, independent of arthropod vector, such as the transmission of infection from dog to dog trough biting or vertical transmission.

- The results of PCR - RFLP and *B. gibsoni* PCR specific molecular diagnostic techniques for in dogs infected with *B. gibsoni* species proved reliable diagnostic methods, identifying dogs microscopically negative.

- The importance of molecular biology investigations in the western part of Romania lies in the fact that the first reference is made to the molecular identification of species *B. canis* in this area. Moreover, this is the first record of the species *B. gibsoni* in Romania confirmed by sequencing, providing important information about the appearance of this species as pathogen in canine population.

- The frequency of clinical signs, evaluated at 20 dogs investigated and diagnosed with acute babesiosis caused by *B. canis*, was 95% for anorexia, 90% for fever, and hemoglobinuria, 70% for the presence of anemic mucosal and 15% for icterus.

- Haematological profile in dogs with babesiosis in this study revealed that the constant changes were eritrocitopenia, decreased hematocrit, thrombocytopenia of varying degrees (severe, moderate) and monocytosis.

- Blood biochemical changes, characteristic of canine babesiosis caused by *B. canis*, were increased bilirubin and aspartate aminotransferase activity.

- Specific medication consisting of a single administration of Imidocarb propionate (Imizol ®, Schering-Plough Animal Health) at a dose of 2.4 mg/kg (0,25 ml/10 kg bw) and the booster administration two weeks later, in all cases, resulted in significant improvement of the health status in a relatively short time (24-48 h), but after administration, in addition to pain, parasimpaticomimetic adverse reactions occurred.

- At the administration of doxycycline hyclat (RONAXAN ®, Merial), at a dose of 10 mg/kg for two weeks with a single daily administration, no side effects were observed and the drug was very well tolerated, however, the recovery measured by the disappearance of the characteristic clinical signs of babesiosis, is late.

This thesis is based on 303 reference titles including 15 web titles and seven titles published as original works of thesis topic.

Key-words: babesiosis, dog, *Babesia canis*, *Babesia gibsoni*.

CONTENT