ABSTRACT

Hepatitis E is an enedemic-epidemic disease, related to the risk of faecal contamination, evolving in many parts of the world where collective hygiene is poor. The disease cartography progresses regularly describing new sporadic cases and epidemic or epidemiologic investigated. Current research shows that regardless of how is evolving hepatitis E in humans (endemic or not), hepatitis E is found in an ubicvitar distribution in pigs farms across the globe.

Thesis entitled "***Research on swine hepatitis E"*** is spread over 212 pages and is made in accordance with current legal provisions, in two main parts: the first part entitled "***Bibliographical study"*** developed in 52 pages, contains 4 tables and 28 figures, part two "***Personal Contribution"*** comprising 112 pages, 21 tables and 93 figures, for better presentation of content.

The first part consists of three chapters in which are summarized information from literature on the subject of the sentence which was subsequently used to interpret and compare data obtained in the second one.

The first chapter entitled "***Bibliographic data on distribution, importance and etiology of hepatitis E***" presents data from literature on the first descriptions of disease and etiological agent, the prevalence and importance of this disease.

Hepatitis E virus (HEV), causal agent of hepatitis E, is an important pathogen responsible for the appearance in many developing countries of a high proportion of acute viral hepatitis in humans, enterically transmitted. The first documentation of disease that developed in India is attested by the existence of 29 000 cases of disease (Purcell and Emerson, 2001) and subsequent frequency of the disease suggests that hepatitis E is a new emerging disease. Hepatitis E was diagnosed in industrialized countries, the initial appearance being associated with cases after traveling in endemic areas, but infections were later diagnosed in persons who had no previous travel in an endemic area. In these cases was demonstrated that the genomic sequence of HEV strains isolated from these patients was more alike to the HEV swine strain prevalent in the population of pigs in the same region, than with human strains. Increasingly more data showed that hepatitis E is a zoonosis and that an animal reservoir for HEV exists. Animal strains of HEV, antigenically and genetically related to human hepatitis E virus were isolated and

characterized from pig (swine hepatitis E virus) and bird (avian hepatitis E virus). Also has been shown that human hepatitis E virus and swine HEV can cause interspecific infection. In addition to pigs and poultry-HEV antibodies have been identified and in other species such as rodents, dogs, cats, sheep, goats, cattle and non-human primates, suggesting that these species have been in contact hepatitis E virus or a related agent. However, to date, pigs are the only species recognized as potential animal reservoir for hepatitis E.

The second chapter entitled "***Epidemiology and pathogenic mechanisms of infection with hepatitis E***" is divided into two parts in which are presented the sources of infection, the transmission mode and resistance to physical and chemical factors, pathogenesis, detailing the viral replication in cell, viral antigens and development of infection.

In the last chapter of the first part entitled "***Symptoms, pathology, diagnosis and control of hepatitis E***", treat issues related symptoms and lesions produced in infections with hepatitis E, the main aspects of diagnosis and control measures.

Swine HEV infection generally occurs at age of 2-3 months and approximately 80-100% of pigs in the U.S. and Japan are infected transitional. Infected pigs have no clinical signs and lesions observed following histopathological examination. In humans, the target population consists of young and middle-aged adults (between 15 and 40 years). Typical symptoms are jaundice, anorexia, nausea, abdominal pain, fever. The mortality rate in humans is 0,2 to 1% in the general population, and up to 15-25% in pregnant women.

Because of asymptomatic hepatitis E in pig, this species is subjected to tests rather than diagnostic screening. From this point of view, research of HEV infection markers is essential in determining endemicity. Searching IgM was sometimes used, but more frequently seroconversion was evidenced by the presence of IgG anti-HEV. The most certain way to detect infection is detect the viral RNA by conventional PCR or real-time. For either method are not commercially specific tests for swine, the methods used were developed based on those used for human genotypes.

Due to ignorance of specific risk factors of sporadic hepatitis E infection is difficult to identify measures to prevent it in non-epidemic areas. It is clear however that in areas where HEV infection is epidemic outbreaks are closely related to contamination of drinking water sources with faeces from infected animals or people. Current data on viral excretion in faeces has

implications for prevention of infection with both epidemic and sporadic nature. Such measures can prevent secondary cases of infection and avoid the risk of transmission to humans.

Because the data regarding replication, pathogenicity and transmission of hepatitis E virus in pigs is incompletely known, preparation of a vaccine or other specific prevention methods has not yet been stipulated. It is considered that after passing through illness, the animal developed neutralizing and protective antibodies.

Chapter IV entitled "***Research on the presence and detection of specific antibodies in farm pigs***" presents the materials and methods used to determine the prevalence of hepatitis E in farm pigs, the diagnosis using two methods: immunoblot (Western blot) and immunoenzymatic assay (ELISA). During the investigations were collected a number of 140 blood samples from seven farms in the counties of Iași and Botoșani, of which 95 sera were tested by Western blot method.

The serological examination of the 95 samples, found to be seropositive to IgG anti-HEV a number of 37 samples, representing 38.94%. The percentage of positivity in the studied farms ranged from 0% in two farms to 84.61%.

Serological tests using enzyme immunoassay tests for hepatitis E in farm pigs aimed to: identify seropositive domestic animals from farms in the counties of Neamț and Brăila and identification of seropositive pigs from import. Of the 62 serum samples, from Braila county Neamț tested by enzyme immunoassay method were identified as HEV positive a number 40, representing 64.51%. Serological examination on four groups of pigs (79 animals) imported from Holland and Spain showed a prevalence of 20.25% of IgG anti-HEV and no group was free from virus.

Chapter V, entitled "***Research on the presence and detection of specific antibodies in pigs from household system***" presents investigations to identify whether infection with hepatitis E is present and to estimate its prevalence. The research was represented of 294 pig's blood samples from households, from which 69 samples were tested by immunoblot method (Western blot) and 170 sera were tested by enzyme immunoassay. Both methods were used to detect the IgG anti-hepatitis E. By immunoblot were tested 69 samples of 10 localities from Iasi county, from which were detected as HEIV positive a number of 34 sera (52.17%), one with dubious results and 24 samples with negative results (34.78%).

Following testing using by enzyme immunoassay 170 serum samples from pigs in five counties in the eastern region of Romania, we identified a number of 33 sera positive for IgG anti-hepatitis E virus, representing a rate of 19.41 %.

In Chapter VI, entitled "***Research on the presence and detection of antibodies to wild boars***" are presented data obtained from research conducted in over two years (2008-2009), during which a number of 110 blood samples of wild animals were collected from two counties: Iasi and Suceava. All serum samples were tested for detection of anti-HEV IgG using immunoenzymatic assay (ELISA HEV). Following the examination were found to be serologically positive for IgG anti-hepatitis E virus a number of five sera, representing a prevalence of 4.54%. The obtained results in this study indicates that Romanian wild boars are infected with hepatitis E virus, although in much smaller proportion than domestic pigs, but it is certain that in the excretory phase they represent a source of virus infection for other animals and for humans.

Chapter VII, entitled "***Research on the correlation of positive serological reactions in animals and humans***" presents results from an epidemiological survey conducted on 67 human samples from different regions of Moldova have been made available for investigation by Public Health Directorate of Iași county and 45 sera from patients with chronic hepatitis type B or C and patients suspected of viral hepatitis in the county of Botoșani. All samples were tested by enzyme immunoassay.

Following the serological exam of 67 sera from counties: Iasi, Suceava, Vaslui, Neamt, Bacău, Botoșani, we identified four positive sera. In Movileni where was identified one HEV-positive sample (serum sample no. 67) in humans, were tested sera collected from nine pigs from households, identifying three anti-HEV IgG positive samples, which may suggest a possible interspecific transmission of infection.

Of the 45 human samples from Botoșani County, by serologic test were detected four positive samples representing 8.88%, while the prevalence of HEV infection in pigs in household system in Botoșani County is 16.94 % and 41.93% in farms (13/31). The correlation of positive serological responses to hepatitis E in the same area both in human and animal draws our attention to the emergence and potential zoonotic risk of infection.

In Chapter VIII, titled "***Investigations on the detection and identification of hepatitis E virus in pigs***" was intended to study the presence of swine hepatitis E virus in farms and to determine the role they play as a reservoir of HEV.

The research on the presence of hepatitis E virus consisted in collection of swine faecal samples and studies on hepatitis E virus viraemia and excretion by age. 14 pig fecal samples were collected from four different units. Faecal samples were collected from shelters (pools) where were housed pigs aged of two and four months. Analyzing the data obtained after amplification of terminal sequence of ORF2 (5996-6343) of hepatitis E virus we identified six positive pools, no farmed being free of infection.

Following the introduction of the two sequences obtained: **EF5** and **FPR4** in BLAST program, were recognized and assigned to sequences of hepatitis E virus genotype 3.

Phylogenetic analysis performed for FPR4/Romania sequence shows that the maximum was 93% homology with hun-E69 strain isolated from human serum of Hungary and HE-JAS3 isolated from human serum in Japan. Recognition and overlapping nucleotide position 100% with genetic homology of 92% was observed from four swine strains isolated in Quebec, Canada.

Regarding the sequence EF5/Romania maximum overlap sequence and the nucleotide recognition with other strains from the database was 97%. Genetic homology with other strains in the terminal sequence of hepatitis E virus ORF2 was 90%, with strain-N6 swJR swine in Japan. A uniformity of 89% presented with strain 8210642 isolated from human serum in southern France. Uniformity of 88% is observed with strains isolated from farm pigs in Mongolia.

Pools were collected from farms where in previous studies we identified positive pigs for IgG anti-hepatitis E. Corroborating the serology results with viral detection we confirming the presence and circulation of hepatitis E virus in farm pigs in Romania. The importance of these results is to emphasize the potential risk of zoonotic transmission of hepatitis E virus strains.