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Rīgas Stradiņa universitāte

Sniedze Laivacuma

IMPROVEMENT OF ECHINOCOCCOSIS DIAGNOSTICS USING ETHIOLOGICAL, BIOCHEMICAL, IMMUNOLOGICAL AND IMMUNOGENETIC MARKERS AND IDENTIFICATION OF INFECTION RISK FACTORS IN LATVIA

Summary of the Doctoral Thesis
for obtaining the degree of a Doctor of Medicine

Specialty – Infectology

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The Doctoral Thesis is available in the RSU library and at RSU webpage: www.rsu.lv.

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ABBREVIATIONS

CE – cystic echinococcosis

CI – 95 % confidence interval

ECDC – *European Centre of Disease Control and Prevention*

GGT – γ- glutamyltransferase

HLA – *human leucocyte antigene*

IL – interleukin

M – mean

max – maximal value

Me – median

Min – minimal value

n – number of patients

OR – odds ratio

p – p-value, probability

Q1- first quartile

Q3 – third quartile

r – correlation coefficient

SD – s*tandard deviation*

SF – Alkaline phosphatase

USS – ultrasound scanning

WHO – World Health Association

INTRODUCTION

Topicality of the study

Echinococcus infection is a parasitosis that is common worldwide. It is traditionally believed that it affects Asian countries more, mostly China, as well as Central Europe and several regions in South America; however, the data from studies conducted in recent years shows that the area of distribution of this infectious disease has expanded. Based on the data monitoring for echinococcosis started in 2003 by the European Union, cases of this infection have been registered in increasingly expanding territories, including the Northern and Eastern regions of Europe (Gottstein, 2010; Giraudoux et al., 2010; Soba et al., 2010). It was the basis for a detailed study of echinococcosis in Latvia.

The disease called "echinococcosis" has two variants: cystic echinococcosis, caused by *Echinococcus granulosus* that from clinical point of view is a less aggressive form of echinococcosis because in most cases it forms clear cut lesions that resemble cysts; while alveolar echinococcosis, caused by *Echinococcus multilocularis*, a disease characterised by an aggressive clinical course, the parasite forms lesions that resemble tumours with infiltrative growth in the damaged organ. Moreover, in the cases of *E. multilocularis* infection, if the parasite has entered the bloodstream, metastasising is possible, thus creating additional problems. A distinction between polycystic or "neotropical" echinococcosis, caused by *Echinococcus vogeli*, and the less common *Echinococcus oligarthus* is made. Both variants mentioned above are characterised by an illness that resembles alveolar echinococcosis but is less aggressive. Until now, these forms of the disease have been registered only in South America. Upon infection of the parasite, mostly the liver is affected, in

rarer cases formation of lesions in the lungs, central nervous system and bones is possible (Eckert et al., 2001).

Timely diagnostics and consequent treatment is frequently too late nowadays as well because at the beginning stage of the disease, there are no specific pronounced clinical manifestations and not infrequently, the diagnosis is confirmed on the basis of ultrasound data, which has been performed due to other medical indications, and only after that the specific serological analysis is carried out. Not infrequently, complaints due to complications have arisen, which encourages specific antibody tests to be performed. If echinococcosis, especially the alveolar form, is not treated, then within a 10-year period after the diagnosis is confirmed, > 90 % cases are lethal (Kern et al., 2003). It is important to note that the course of echinococcosis, if not discovered and radically treated in time, is chronic and requires substantial financial resources because the antiparasitic treatment often must be administered for life, to stop the spread of the infection (Eckert et al., 2001).

The incidence of echinococcosis in the European countries varies from 0.1 to 10 cases per 100,000 residents (Gottstein, 2010; Giraudoux et al., 2010; Soba et al., 2010), the number of cases of echinococcosis diagnosed for the first time is rather high in Latvia as well, despite comparatively low population figures. The location of Latvia next to endemic regions for this zoonotic disease, for instance, Russia, Belarus, Poland is also an important factor; however, no targeted studies on the incidence, risk factors, diagnostics and therapy have been conducted particularly in our country; data on the quality of life of patients in the cases when radical surgical extraction of the parasite was impossible and years of medication therapy are required, are also lacking.

Data on the use of immunological parameters, mainly IL-4, IL-10, in addition to radiological and serological methods of diagnostics for evaluating the effectiveness of the treatment and specifying its further treatment tactics is

available in literature; however, the data is not detailed enough (Nouir et al., 2008; Rigano et al., 1995).

It is therefore important to summarise and analyse data on the prevalence and course of echinococcosis to find options for early diagnostics and radical therapy.

Aim of the study

To determine, summarise and analyse the prevalence of echinococcosis in Latvia, to clarify the course of the disease, to improve diagnostic and treatment tactics and to identify the risk factors of the disease.

Study objectives

1. To analyse population data to determine distribution of echinococcosis in Latvia.
2. To identify risk factors, including immunogenetic that can promote infection with *Echinococcus sp.*
3. To analyse and compare ultrasonoscopy and serological investigation data prior to treatment and at selected time points.
4. To identify if immunological markers - IL-4, IL-10 can be used to guide treatment length.
5. To compile and analyse the obtained data and to prepare recommendations for doctors to improve the diagnosis and treatment of echinococcosis.

Hypothesis

1. Echinococcosis patients are found practically throughout all Latvia.
2. Diagnosis and treatment of echinococcosis is in most cases delayed and can be influenced by the identification of risk factors to improve disease prevention.
3. The efficiency of the diagnosis and monitoring of echinococcosis can be improved by regular radiological and serological tests and immunological markers (IL-4, IL-10).
4. Immunogenetic studies may show protective and predisposing alleles.

Novelty of the Thesis

In this study, a relationship between specific biochemical, immunogenetic markers and radiological characteristics and progression and complexity of the disease and its association with the characteristics of treatment was verified; this will allow one:

1) to set up suggestions for practising doctors for optimising the examination and treatment of patients;
2) to plan the duration of pharmacological therapy and partially predict its effectiveness.

The above mentioned would improve the healthcare for patients with echinococcosis and the use of financial resources.

Meanwhile, the identification of the risk factors and detection of protective and predisposing alleles defined in the immunogenetic tests would allow one to survey the patients of this group and perform a targeted examination to diagnose this disease as early as possible.

Ethical aspects of work

On November 29, 2012, the permission of the Ethics Committee of Rīga Stradiņš University was received for the research (see appendix).

1. MATERIAL AND METHODS

1.1. Patient groups

The study analysed the medical documentation available for 144 patients with echinococcosis in RAKUS and PSKUS for the period from 1 January 1999 to 1 February 2015.

The following indicators were used as inclusion criteria based on available worldwide recommendations:

1) serological confirmation of echinococcosis;
2) radiologically (at least USG) confirmation of echinococcosis;
3) one of the organs affected by the parasitosis is the liver.

During the selection process, 28 patients were not included in the study because the diagnosis cannot be considered verified if there are only positive serological tests since there might be cross-reactions with other cestode parasites. Also other patients were excluded from the study group because simple cysts (radiologically not typical of echinococcosis) finding in the liver cannot be considered to be echinococcosis if not confirmed by serological tests.

Thus, 116 patients were enrolled in the study for whom the first time diagnosis of echinococcosis was confirmed between January 1, 1999 and February 1, 2015. It can be considered as a general group (n = 116) within the framework of the doctoral thesis because in the above-mentioned medical institutions information about all patients with echinococcosis in Latvia during this period was gathered.

Of the 116 patients included, 87 were women and 29 were men with an average age of 54.3 years (SD ± 16.2). *Echinococcus granulosus* infection was verified in 87 patients and 29 in *Echinococcus multilocularis* infection.

1.2. Questionnaires about risk factors

In order to ascertain the risk factors of the disease, a questionnaire was carried out on echinococcosis and echinococcosis-free patients on possible risk factors for the disease. Of the 116 Latvian echinococcosis patients whose data were used for the initial analysis, 31 patients died at the time of the questionnaire, thus 85 echinococcosis patients were only invited to participate in the survey. Thirty-eight patients did not wish to attend the questionnaire for various reasons; therefore information on potential risk factors was obtained from 47 patients. Assuming that there are 85 patients in the general population, the sample error was 9.8 %, with a 95 % confidence interval. The resulting error exceeds the usual 5 % threshold, but does not exceed 10 %, so the resulting sample could be attributed to the entire general population.

The criteria for the inclusion was:

1) a resident of Latvia for at least 15 years and alive at inclusion;
2) was diagnosed with echinococcosis by combination of positive serological test result and diagnostic imaging findings;
3) diagnosis was established between 1999 and 2015;
4) echinococcosis has affected the liver.

For each case, a control was selected that was matched by gender, date of birth (± 5 years), principal place of residence (district level) during the previous 10 years. The controls had to fulfill the following inclusion criteria:

1) a resident of Latvia for at least 15 years;
2) had never been diagnosed with CE;
3) had an ultrasound examination of the abdominal cavity performed within the last five years without significant pathology detected.

Individuals from the control group were selected from family doctor and infectologist practices from different regions of Latvia to represent the

participants in all statistical regions of Latvia. All subjects in the control group signed a voluntary agreement to participate in the study.

The questionnaire consisted of 45 questions, divided into the following groups:

1) personal date (sex, date of birth and current place of residence);
2) living conditions of the patient (apartment, country home equipped with or without animal pen, etc.);
3) occupation (weather or not related to agriculture and livestock farming involving the various animal species);
4) ownership of or contact with dogs (number of animals owned per life time, time period, living conditions, animal behavior, ingestion of viscera and/or wild animals, deworming);
5) having owned farm animals (animal species, duration, housing conditions, slaughtering conditions);
6) having household garden (type of garden, duration, plants grown, fertilising);
7) hunters in the family;
8) berry and mushroom gathering habits;
9) travel history.

Since it was difficult to ensure that interviewer would be ignorant to the interviewee`s case or control status, questions were phrased in such a way that the replies had to be quantitative or carefully selected from series of mutually exclusive options that covered all possible answers and avoided subjectivity both in the replies and in the interpretation put upon these by the interviewer.

1.3. Laboratory diagnostics

In this study, specific and non-specific laboratory tests were performed at the National Microbiology Reference Laboratory (Riga East University Hospital) and The Joint Laboratory of Clinical Immunology and Immunogenetics (Rīga Stradiņš University). The assessment of the obtained results was performed in accordance with the annotations by test system manufacturers.

1.4. Confirmation of echinococcosis

Detection of specific antibodies was used, either against *Echinococcus sp.* with an ELISA method or against *Echinococcus granulosus* by agglutination reaction. The Immunoblot method was used as a confirmatory method for determining specific IgG class antibodies to *Ecinococcus spp.* (possibility to identify *Echinococcus granulosus* and *Echinococcus multilocularis*).

***Echinococcus sp.* IgG**: Antibodies against *Echinococcus sp.* detected by using ELISA. Used test system: Novagnost *Echinococcus* IgG, Nova Tec Immundiagnostica GmbH, Germany / Ridascreen *Echinococcus* IgG, R-biopharm, Germany.

***Echinococcus granulosus* IgG**: Antibodies against *Echinococcus granulosus* detected by using agglutination reaction (indirect hemagglutination). Used test system: *Echinococcus* Serodiagnosis of hydatidosis by indirect haemagglutination; ELITech MICROBIO, France.

***Echinococcus sp.* IgG**: Antibodies against *Echinococcus sp.* detected by using *Immunoblot* method. Used test system: ECHINOCOCCUS Western Blot IgG. Immunoblot asssay for in vitro diagnostic use. LDBIO ECHINOCOCCUS Western Blot IgG., LDBIO Diagnostics, France.

1.5. Detection of cytokines

Detection of cytokines (IL-4, IL-10) concentration was performed in the RSU Clinical Immunology and Immunogenetic Interdepartmental Laboratory.

Blood serum from the subject was used to determine cytokine levels. Venous blood was obtained by venopuncture using BD (Becton Dickenson) vaccinia. The patient material was centrifuged after extraction, the serum divided into eppendorf tubes and frozen at −70 °C until the test.

IL-4, IL-10 level determined in the patient's test material using standard ELISA (immuno-fermentation method) using *Vector-Best* test system, Russia.

IFA-BEST (IL-4, IL-10) was detected by the microplate solid phase immuno-fermentative method. The test used monoclonal antibodies against various epitopes. Calibrators and test patient material (100 µl serum) react with monoclonal antibodies (MKA1) that cover the microplate and react with monoclonal antibodies conjugated with peroxidase (100 µl MKA2). After incubation, complex MKA1 – human (IL-4, IL-10) – MKA2-peroxidase is formed. After incubation, the microplate is washed to separate the unbound antibodies. Add the chromogen (TMB), incubate for 25 minutes and stop the reaction with the Stop-stop solution. Read the result with a photometer (Biotec Fluorescence Microplate Readers) using a defined wavelength of 450 nm. The photocalorimetric reading of the optical density is directly proportional to the concentration of IL in the test material.

After calibration of the calibrators, a calibration curve is obtained from which the results of the test material (IL-4, IL-10) are read. IL-4 – measuring range: 0–100 pg/ml; sensitivity: 0.4 pg/ml; IL-10 – measuring range: 0–500 pg/ml; sensitivity: 0.1 pg/ml.

1.6. Detection of immunogenetic factors

HLA-DRB1; DQA1; AQB1 typing detection were performed in Riga Stradinš University Joint Laboratory of Clinical Immunology and Immunogenetic. For HLA Class II alleles detection 4 ml of peripheral blood with EDTA were collected and stored at −20 °C before detection. For human DNA extraction QIAamp® DNA Blood Kit (Germany) was used in accordance with the methodology approved by the manufacturer**.**

DNA amplification was performed by Real-Time polymerase chain reaction (RT-PCR) with low resolution sequence specific primers (DNA-Technology, Russia) according to manufacturer's instructions (Anonymous, 2018). Each kit contained an internal control for evaluation of RT- PCR quality and positive control. HLA typing included identification of HLA-DRB1* alleles (01 to 18), HLA-DQA1*alleles (01:01, 01:02; 01:03; 01:04, 02:01; 03:01;04:01, 05:01, 06:01), and HLA-DQB1* alleles (02:01-02:02; 03:01-04; 04:01-04:02; 05:01-04; 05:02-03; 06:01; 06:02-08).

Material from the data base of the Joint Laboratory of Clinical Immunology and Immunogenetics of RSU was used for the comparison of HLA test results, respectively, HLA of healthy blood donors (n = 100) was determined at the laboratory.

We used term "complicated" or severe course of the disease which can be defined by following criteria:

1) echinococcosis lesion larger than 10 cm;
2) lesion count 5 or more;
3) inability to use radical treatment;
4) reoccurrence of the disease after surgical removal of the parasite.

1.7. Utrasound evaluation

Considering that only ultrasound description data were available, cystic echinococcosis stages were determined based on these data. Stages were determined based on WHO recommendations. The stage was marked at the time of diagnosis and then every following year.

Table 1.1

Classification of cystic echinococcosis using ultrasound

Stage	Characteristics	Notes
CL	• Status: fertile cyst • Unilocular cysic lesion with uniform anehoic content • Cyst wall not demarked • Round or oval • Size: CLs (< 5 cm), CLm (5–10 cm), CLl (> 10 cm)	Usually non-parasiticcysts but might be early stage echinococcal cysts No pathognomic signs. Additional diagnostic methods needed.
CE1	• Status: fertile cyst • Unilocular cysic lesion with uniform anehoic content. “Snowflake sign” because of capsule movement. • Cyst wall usually not well demarked • Round or oval • Size: CE1s (< 5 cm), CE1m (5–10 cm), CE1l (> 10 cm).	Pathognomic signs – cyst wall and “snowflake sign”
CE2	• Status: fertile cyst • Multivesicular, multisepteted cyst, partially or totally filled with daughter cysts. “Rosette sign” or “honeycomb sign” might be present. • Cyst wall well demarked. • Round or oval • Size: CE2s (< 5 cm), CE2m (5–10 cm), CE2l (> 10 cm)	Pathognomic signs.

The end of table 1.1

Stage	Characteristics	Notes
CE3	• Status: Transitional • Anechoic contents – "waterlilly sign" • Unilocular cyst, might contain daughter cysts (anechoic) and hyperechoic areas (mambranes, degenerative daughter cysts). Complex masses. • Different shapes. • Size: CE3s (< 5 cm), CE3m (5–10 cm), CE3l (> 10 cm).	Pathognomic signs – detached cystic wall.
CE4	• Status: Infertile • Heterogenous, non-homogenous contents. No daughter cysts. • "Ball of wool" sign • Size: CE4s (< 5 cm), CE4m (5–10 cm), CE4l (> 10 cm).	Usually signs not pathognomic. Additional diagnostic methods needed.
CE5	• Status: Infertile • Thick calcified walls, arch-shaped wall. Produce a cone shaped shadow. • Totally or partially calcified. • Size: CE5s (< 5 cm), CE5m (5–10 cm), CE5l (> 10 cm)	Diagnosis might be unclear. Some signs are pathognomic.

1.8. Statistical analysis of the data

Descriptive statistical methods were used to characterise patients. Quantitative data were evaluated for mean arithmetic (Mean, M) with standard deviation (SD), but if data did not correspond to normal distribution – Median and Interquartile Range (IQR).

Results were evaluated with 5 % α-error, so if the p-value was less than 0.05, the zero hypothesis was rejected, and the test result was considered statistically significant.

Pearson's chi-squared test ($\chi 2$) was used to assess the qualitative differences between patient groups (both study groups and control groups). In addition to the 2×2 tables, Fisher's exact test was used if the expected count of any 2×2 table cell was less than 5. To avoid a false positive result, we used

Bonferroni's correction. We used Mantel-Haenszel or Yates correction methods to assess risk heterogeneity.

Correlation analysis was used to determine the relationship between the variables, Spearman's rank correlation coefficient (rs). Cox proportional-hazards regression was used to assess the factors that affect patient survival.

Data processing was performed using IBM SPSS Statistics, Version 22.0.

2. RESULTS

2.1. Epidemiological data

Data on the period from 1999 to February 2015 was analysed. Upon the assessment of the distribution of newly diagnosed cases over the years, it may be established that overall the number of new cases is fluctuating; however, a tendency is observed that after 2004 the number of new cases tends to increase (Figure 2.1).

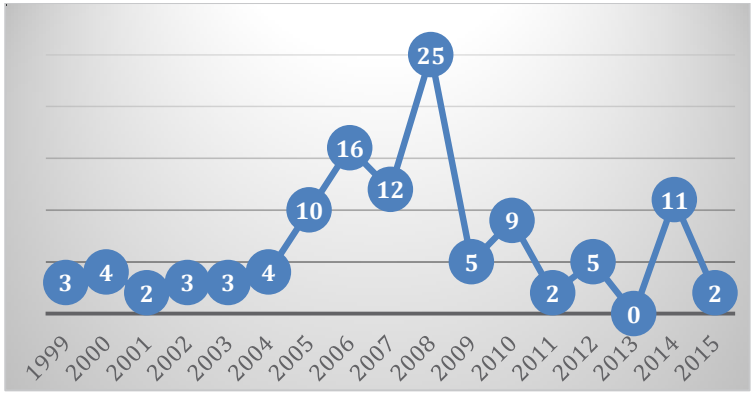

Figure 2.1 **Number of confirmed echinococcosis cases**

Overall, a group of 116 patients were analysed, where a convincing majority were female – 87 (75 %) and only 29 (25 %) were male (Figure 2.2).

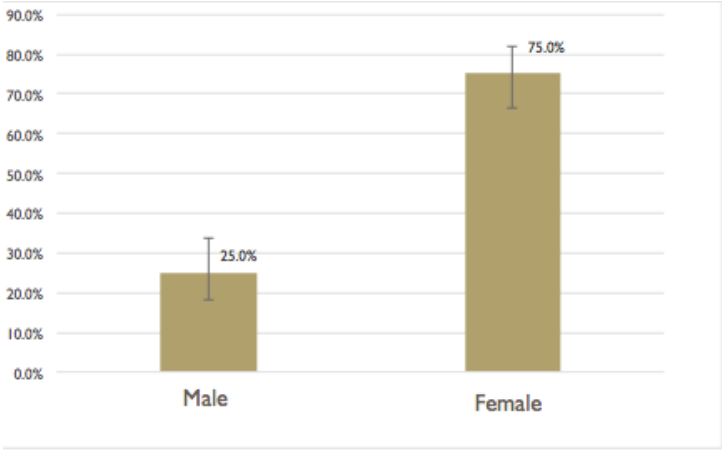

Figure 2.2 **Gender of echinococcosis patients**

In this study, the residence where patients had lived more than 15 years was analysed; Figure 2.3 shows that a large number of patients lived in Kurzeme region and several in Latgale – closer to Lithuania where the number of this parasitosis each year is comparatively high. The number of patients in Riga is also comparatively high; however, in those cases presumably the infection has not acquired there, but in the regions. Comparatively few patients are from Vidzeme region, which is closer to Estonia where the number of cases is very low.

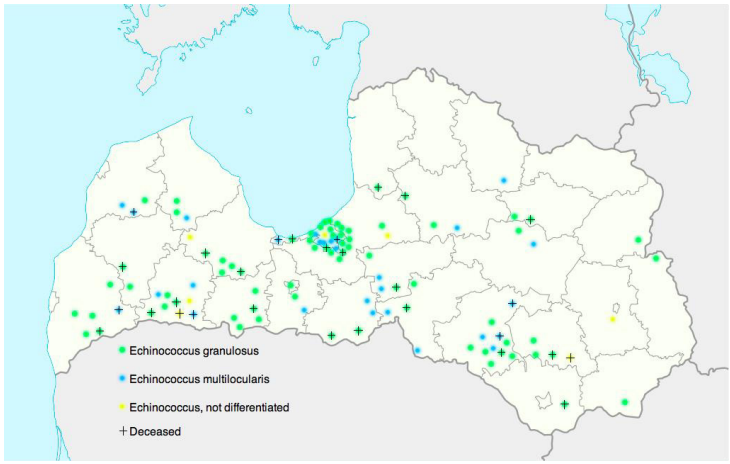

Figure 2.3 **Echinococcus patient distribution, Latvia**

Mean age upon diagnosis was 54.3 years (SD ± 16.2), the age distribution corresponds to normal distribution (Figure 2.4).

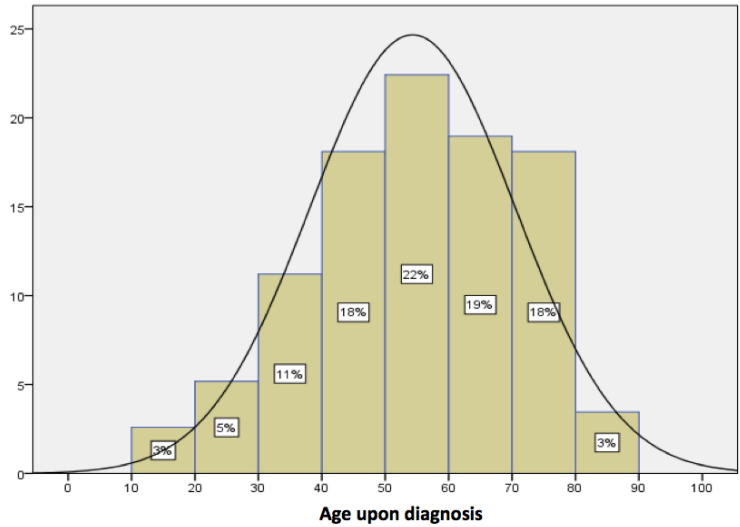

Figure 2.4 **Age of echinococcosis patients**

Comparing the age of the patient upon diagnosis, the age difference between male and female was not statistically significant ($p = 0.219$).

In this study, 12 most frequent complaints, which are described in the available literature as well, were chosen for analysis. The complaints were variable, but in most cases associated with the gastrointestinal system: most frequently 73 (62.9 %) patients mentioned itching skin, 29 (25.0 %) abdominal discomfort but 24 (20.7 %) excessive fatigue (Table 2.1).

Table 2.1

Initial complaints of patients (n = 116)

Complaint	n_1	Frequency, %	95 % CI^-	95 % CI^+
Pruritus	73	62.9	53,9 %	71.2 %
Anorexia	11	9.5	5.4 %	16.2 %
Nausea, vomiting, diarrhea	2	1.7	0.5 %	6.1 %
Abdominal pain	5	4.3	1.9 %	9.7 %
Abdominal discomfort	29	25.0	18.0 %	33.6 %
Fatigue	24	20.7	14.3 %	28.9 %
Bitter taste	9	7.8	4.1 %	14.1 %
Burning sensation in the abdomen	3	2.6	0.9 %	7.3 %
Jaundice	1	0.9	0.2 %	4.7 %
Weight loss	6	5.2	2.4 %	10.8 %
Back pain	1	0.9	0.2 %	4.7 %
Cough	3	2.6	0.9 %	7.3 %

n_1 – number with patients with mentioned complaint
CI – confidence interval

Analysing the nature of complaints that may be associated with the presence of infection and age of the patient, it is evident that stomach pain is more typical for older patients ($r_s = 0.189$, $p = 0.042$), whereas excessive fatigue is typical for younger patients ($r_s = -206$, $p = 0.026$).

Analysing the nature of complaints and the age of patients, statistically significant differences were not found.

2.2. Diagnostic characteristics

In the case of echinococcosis, the approach should be based on 2 parameters – serological data and ultrasound findings. Usually, first the radiological examination was performed, namely, ultrasound, and then diagnosis was confirmed with the serological method.

Analysing the initial ultrasound description for 113 patients (echinococcosis foci were not found in the liver in 3 patients), in the initial conclusion echinococcosis was verified only in 6 (5.3 %) cases, most frequently the conclusion was "liver lesion" – in 46 (40.7 %) cases (Table 2.2).

Table 2.2

Initial interpretation of the ultrasound

Initial interpretation	n_1	n	Frequency, %	95 % CI^-	95 % CI^+
Liver lesion	46	113	40.7	32.1 %	49.9 %
Metastasis	1	113	0.9	0.2 %	4.8 %
Hemangioma	16	113	14.2	8.9 %	21.8 %
Abscess	2	113	1.8	0.5 %	6.2 %
Cyst	25	113	22.1	15.5 %	30.6 %
Ehinococcus	6	113	5.3	2.5 %	11.1 %
Tumor	18	113	15.9	10.3 %	23.8 %
No interpretation	3	116	2.6	0.9 %	7.3 %

n_1 – number of patients with mentioned sign
n – total number of patients

Overall, 80 out of 116 patients were diagnosed with an *Echinococcus granulosus* infection and 29 patients were diagnosed with an *Echinococcus multilocularis* infection, in 7 cases it was not possible to serologically identify species of the parasite (Table 2.3).

Table 2.3

Serological verification of echinococcosis (n = 116)

***Echinococcus* species**	**n_1**	**Frequency, %**	**95 % CI$^-$**	**95 % CI$^+$**
E. granulosus	80	69.0	60.1 %	76.7 %
E. multilocularis	29	25.0	18.0 %	33.6 %
Not possible to differentiate	7	6.0	3.0 %	11.9 %

n_1 – number of patients with mentioned sign

Analysing the possible relationship between the initial suspicion of echinococcosis (according to the ultrasound) and serological confirmation, it was concluded that in the cases when it was not possible to differentiate the subtype of the parasite serologically, more frequently no formations were differentiated according to the initial suspicion and more frequently the term "liver lesion" was used ($r_s = 0.205$, $p = 0.029$).

Upon assessment of the obtained data reflected in ultrasounds, it can be concluded that for the majority of patients initially (upon diagnosis) the liver is normal in size, with a tendency to decrease over time; however, the number of patients that have undergone a surgery and mostly the right lobe of the liver is involved in the pathological process is increasing, but the involvement of both liver lobes tends to increase when the course of disease is progressing, the number of foci tends to increase (however, it must be noted that the period of time chosen for the study is comparatively short because a large number of the patients lack examinations over time). There are no convincing changes in the size of the focus over time. In this study, it was found that as the duration of observation increases and the disease progresses, the number of patients who had bile ducts and blood vessels involved in the parasitic process increases.

It was also evaluated whether there was a relationship between any of the echinococcosis species and the most characteristic USG findings (according to liver size, lobe involvement, number of foci, size); however, it was not found.

Analysing the relationship between the age of the patient at the time of diagnosis and characteristics found in ultrasounds, it was found that the older the patient, the higher the number of foci ($r_s = 0.215$, $p = 0.027$).

Analysing the association that was found between the nature of complaints and USG findings, it was found that if the focus was in the left lobe of the liver, patients complained about bitterness in mouth more frequently. ($r_s = 0.365$, $p < 0.001$).

2.3. Treatment data

In order to assess and analyse the treatment tactic in the case of confirmed echinococcosis, the characteristics of individually received therapy were analysed. Further in the text, a concept of etiotropic "therapy course" is used, which means that therapy is received for at least 30 days.

It was found that most patients received etiotropic therapy within one year after diagnosis – 82 (70.7 %) patients. In contrast, analysing the compliance with the available recommendations, it was found that only 9 (7.8 %) patients received therapy for at least 6 months during the 1st year of observation. Upon finding out this fact, the analysis was continued with a purpose to ascertain whether each patient has ever had a therapy course of 6 months, and it was determined that only 18 (15.5 %) patients had had such a therapy course. Data analysis showed that etiotropic therapy was administered to only 58 (50.0 %) patients.

Assessing the above-mentioned parameters in context with the type of the parasite, a correlation between the type of the parasite – *Echinococcus multilocularis* and therapy regimen – at least 6 months in 1 year of therapy was found ($r_s = 0.205$, $p = 0.028$). However, analysing those patients, no statistically significant correlation was found with the fact that they would receive therapy

every year (r_s = −0.085, p = 0.362). No statistically significant correlation between *Echinococcus granulosus* and parameters of therapy was found.

Analysing the number of therapy courses of one individual patient during the observation period, it can be concluded that the mean arithmetic of the overall number of therapy courses was 6.0 (SD ± 8.3), with the median 2. The minimum of received courses was 0, hence, no therapy courses were received, but the maximum was 43 therapy courses.

Assessing the available data, it can be concluded that overall the median of the duration of received therapy is 1 year ($p < 0.001$), which is considerably less than expected.

Assessing whether there is a correlation between how many years the patient has been observed and received therapy, a statistically significant correlation is found ($r_s = 0.604$, $p < 0.001$); therefore, the duration of therapy increases if the duration of observation is prolonged. A statistically significant correlation between how long the patient is under observation and the overall number of therapy courses as well; therefore, the longer the observation period, the more therapy courses the patient will have received ($r_s = 0.51$, $p < 0.001$).

The analysis of serological results that was described before, associating the effectiveness of etiotropic therapy with a specific parameter of the disease is important – specific antibodies to *Echinococcus* as such and its species. Results of the analysis showed that a positive effect was found in approximately half – the titre decreased in 31 (26.7 %) patients, but the antibodies disappeared completely in 25 (21.6 %) patients.

Assessing the changes in titre, it can be concluded that in the cases when therapy has been administered within one year after confirming diagnosis, the chances that the antibodies will disappear are higher ($p < 0.001$) (Figure 2.5).

In addition, if therapy has been received every year, the chances that the antibodies will disappear are higher ($p < 0.001$) (Figure 2.6).

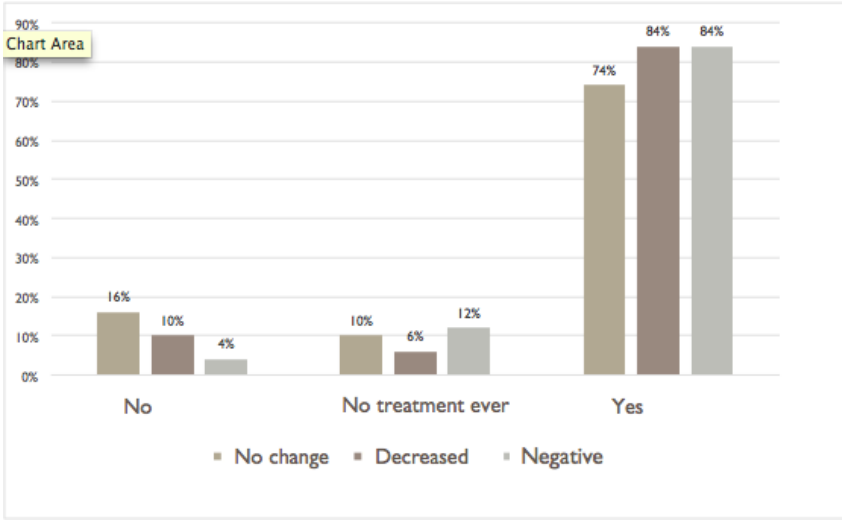

Figure 2.5 **Serological findings for patients who received treatment during first year after diagnosis**

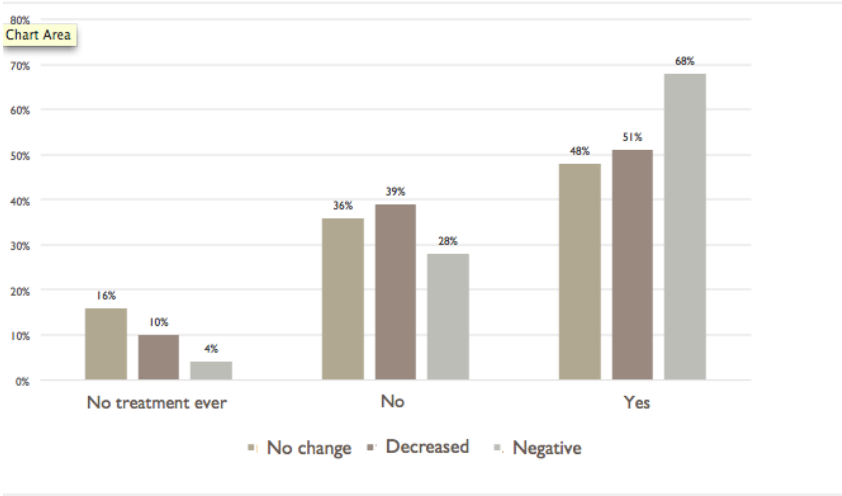

Figure 2.6 **Serological findings for patients who received treatment every year**

It is important that the relationship can be seen – as the duration of therapy increases, the possibility that the antibodies will disappear increases ($p < 0.001$). Also, as the number of therapy courses increases, the possibility that the antibodies will disappear increases ($p < 0.001$).

Analysing the correlation between the stage of echinococcosis upon diagnosis and at the end of the observation period, it can be concluded that the stages increase approximately by 0.2 points. Comparing the treated and untreated patients, it was observed that in treated patients the stage of the infection tends to progress ($p = 0.011$), compared to the untreated, in which the stage did not change (Table 2.4).

Table 2.4

Change in stages in patients with and wihout treatment

Patient group	Stage of echinococcosis	M	Q_1	Me	Q_3
Treated patients	Echinococcosis stage in beginning of observation	3.3	2.8	3.5	4
	Echinococcosis stage at the end of observation	3.3	2.8	3.5	4
Patients without treatment	Echinococcosis stage in beginning of observation	**2.6**	2	2	3
	Echinococcosis stage at the end of observation	**2.8**	2	3	3

M – mean arithmetic
Me – median
Q_1 – firts quartile
Q_3 – third quartile

2.4. Analysis of immunological markers

In this study, immunological markers IL-4 and IL-10 were evaluated as well. The IL-10 level in different patients fluctuated between 0–507.5 pg/ml. The IL-4 level in all patients was below the detection limit. A statistically significant correlation with elevated IL-10 levels and a more severe course of disease in the case of alveolar echinococcosis ($r_s = 0.315$, $p = 0.031$) was observed.

2.5. Analysis of risk factors

2.5.1. Analysis of epidemiological risk factors

Cases and control patients did not show significant differences between the age groups. The youngest patient was a 24-year-old man, while the oldest patient was an 84-year-old woman. The number of women was significantly higher than the number of men ($p < 0.05$).

When analyzing the association of echinococcosis prevalence in cases and control we concluded that:

1) patient groups with dogs, compared to those who had never had dogs or keep them in controlled conditions, the risk of echinococcosis was significantly higher (OR 6.526, $p < 0.01$) among those persons whose dogs were kept in kennels or leashes near dwelling;
2) the factor "living conditions of dogs" showed a significant positive correlation with "preying of wild animals and/or feeding with viscera" ($r = 0.754$; $p < 0.01$) stating that dogs free to roam may have higher possibility to praying on wild animals and/or viscera;
3) patients groups with cats, compared to those who had never had cats or has only indoor cats, the risk of echinococcosis was significantly higher (OR 3.325, $p < 0.05$) among those persons whose cats were free to roam;
4) the factor "living condition of cats" showed a significant positive correlation with "preying of wild animals and/or feeding with viscera" ($r = 0.717$; $p < 0.01$) stating that cats free to may have higher possibility to prey on wild animals and/or viscera;
5) risk of echinococcosis was significantly higher for those who always have close contact with dogs/cats (OR 6.500, $p < 0.05$);

6) risk was significantly higher to those who lived in rural dwelling (OR 57.646, $p < 0.01$);
7) risk was significantly higher to those who have hunters in family (OR 4.498, $p < 0.05$).

The results of present study show that having a household garden, or gathering mushrooms or berries posed no excess risk of disease.

2.5.2. Analysis of genetic risks factors

A quantitative analysis of DRB1, DQA1 and DQB1 alleles of HLA Class II were done during immunogenetic testing in all patients with echinococcosis, as well as separately in cystic and alveolar echinococcosis groups.

The predisposition to develop the cystic echinococcosis in the patient group was associated with the HLA-DRB1*17:01 (OR = 4.63; $p < 0.000$), HLA-DRB1*07:01 (OR 5.65; $p < 0.004$) alleles. Moreover, the allele -DRB1*15:01 (OR = 0.19; $p = 0.001$) was rarer in cystic echinococcosis in the patient group and significantly more frequent in controls. For DRB1*01:01, DRB1*08:01 and -DRB1*13:01 alleles detected HLA-DRB1 differences were not significant after Fisher's correction. Probably, only DRB1*15:01 has a protective effect in the pathogenesis of cystic echinococcosis.

The alveolar echinococcosis in patients group showed association with the HLA-DRB1*17:01 (OR = 3.07; $p = 0.033$), DRB1*07:01 (OR = 7.0; $p = 0.014$) alleles, but for DRB1*11:01 allele this detected HLA-DRB1 differences were not significant after Fisher correction. Probably, only HLA-DRB1*17:01 and DRB1*07:01 alleles have a predisposition effect in the pathogenesis of alveolar echinococcosis in the patient group. The alleles -DRB1*13:01 (OR = 0.39; $p = 0.591$), DRB1*15:01 (OR = 0.23; $p = 0.106$)

and -DRB1*01:01 (OR = 0.78; p = 0.545) was rarer in alveolar echinococcosis in the patient group and significantly more frequent in controls, but differences were not significant after Fisher's correction.

In all patients allele combinations *04:01/*11:01 (OR = 8.75, p = 0,002), *17:01/*13:01 (OR = 8.11, p = 0,037) and *11:01/*13:01 (OR = 4.90, p = 0,044) were associated with more severe diasease presentation. In cystic echinococcosis patients group allele combinations *04:01/*11:01 (OR = 7.78, p = 0.010) lead to more complicated disease presentation. Also in alveolar echinococcosis group the severity of diasease was linked to three allele combinations *17:01/*13:01 (OR = 16.33, p = 0,001). No protective alleles in this group were found.

The predisposition to the cystic echinococcosis patients group was associated with the HLA-DQB1*03:02 (OR = 3.67; p = 0.02), HLA-DQB1*03:01 (OR = 2.01; p = 0. 033). On the other hand, allele -DQB1*05:01 (OR = 0.25; p = 0.047), DQB1*06:02-8 (OR = 0.31; p = 0.013) was rarer in cystic echinococcosis in the patient group. It may be assumed that HLA-DQB1*05:01 and DRB1*06:02-8 have a protective effect in the pathogenesis of cystic echinococcosis in the patient group rather than in the control group (gf = 0.12 and 0.23).

The alveolar echinococcosis in the patient group showed association with the HLA-DQB1*05:01 (OR = 3.18; p = 0.037) alleles. Probably, only these alleles have a predisposition effect in the pathogenesis of alveolar echinococcosis. The alleles DQB1*03:02 (OR = 8.03; p = 0.643), DQB1*04:01-2 (OR = 11.48; p = 0.545) and DQB1*06:02-8 (OR = 1.49; p = 0.091) were rarer in alveolar echinococcosis patient group and more frequent in controls, but differences were not significant after Fisher's correction.

While analyzing HLA-DQB1 allele group, it was found that *03:01/*03:02 (OR = 17.68, p = 0.0004), *02:01-2/*03:01 (OR = 3.84,

p = 0.047) and *03:02/*06:02-8 (OR = 4.02, p = 0.08) allele combinations were linked with severe clinical symptoms in all groups.

Regarding allele combinations, it was concluded that in cystic echinococcosis group the following genotypes lead to a more severe disease: *02:01-2/*03:01 (OR = 5.17, p = 0.024), *03:01/*03:02 (OR = 33.00, p = 0.014) and *03:02/*06:02-8 (OR = 5.94, p = 0.042) but in alveolar echinococcosis group *03:01/*03:02 (OR = 25.83, p = 0.0004).

HLA-DQA1 group alleles were also analyzed, which allows to conclude that in cystic echinococcosis group the most frequent one was and possibly indicating higher risk for more severe course of the disease was allele *04:01 (OR = 3.84, p = 0.006) but allele *01:02 (OR = 0.28, p = 0.013) was rare and possibly protecive. Also alleles *01:03 (OR = 1.81, p = 0.181) and *02:01 (OR = 1.69, p = 0.204) were less frequent than in the control group but not statistically significant.

In this allele group for alveolar echinococcosis patients it was found that alleles *01:01 (OR = 1.89, p = 0.291) and *03:01 (OR = 1.48, p = 0.388) were rarer than in control group and possibly protective but it was not statistically significant.

It was possible to link *01:03/05:01 (OR = 5.65, p = 0.040) and *03:01/05:01 (OR = 3.47, p = 0.031) with severe diasese course in all groups and also in alveolar echinoccosis group *03:01/*05:01 (OR = 4.23, p = 0.016) and just 01:01/02:01 (OR = 10.78, p = 0.044) in alveolar echinoccosis group.

Echinococcus infection have association with genotypes presented in Table 2.5.

Table 2.5

Three locus - DRB1/DQB1/DQA1 – allele distribution in echinococcosis and control groups

Group haplotypes	**All patients (n = 42)** Gf/OR(p)	**Cystic echinococcosis patients(n = 29)** Gf/OR(p)	**Alveolar echinococcosis patients (n = 16)** Gf/OR (p)	**Control group (n = 100)** Gf
***11:01/*03:01/*05:01**	**0.22/2.79 (0.047)**	**0.28/3.85 (0.015)**	–	0.09
***15:01/*06:02-8/*05:01**	**0.03/0.15 (0.027)**	**0.03/0.19 (0.064)**	–	0.16
***04:01/*03:01/*03:01**	0.22/6.62(0.001)	**0.28/9.14 (0.001)**	–	0.04
***13:01/*02:01-2/*05:01**	0.03/0.19 (0.065)	**0.03/0.24 (0.128)**	–	0.13
***17:01/*02:01-2/*01:01**	0.16/6.26 (0.006)	–	**0.75/97.0 (0.000)**	0.03
***11:01/*03:01/*01:03**	0.11	0.05	**0.17/20.63 (0.0001)**	0.01
***11:01/03:01/*03:01**	0.19	0.03	**0.63/165 (0.000)**	0.01

Green – protective
Red – more severe disease
n – number of patients
OR – odds ratio
p – probability
GF – gene frequency

2.6. Mortality of included patients

In this study, it was concluded that 31 out of 116 patients died, 13 of which the cause of death was echinococcosis, the death of 5 patients may be associated with echinococcosis, as the cause of death was associated with liver pathology. Among other causes of death were malignancies (prostate cancer, colon or rectum cancer, lymphoma, lung cancer, ovarian cancer), other infectious diseases (pneumonia, spondylitis), ulcer disease, and atherosclerosis.

3. DISCUSSION

In this study, 116 patients diagnosed with echinococcosis for the first time from 1999 to February 2015 were included. Analysing the number of newly registered cases, a tendency can be seen that starting from 2005, the number of newly registered cases increases, reaching the highest level in 2008 – in one year, 25 cases were diagnosed, then the number decreased, stabilising at 5–10 cases each year. Presumably, the increased number of cases may be associated with the emergence of new serological methods of diagnostics and improved accessibility to radiological examinations, as well as improved knowledge of medical professionals about this parasitosis. Based on the data published by the ECDC (European Centre for Disease Prevention and Control), it can be concluded that echinococcosis in Latvia can be regarded as a rare pathology, compared to the available data about Europe. Compared to other Baltic states, Latvia can be ranked in the middle because the number of cases in Lithuania fluctuates between 12–33 a year, but there are no new cases in Estonia in most of the years inspected, except 2007 when there were 2 new cases and in 2013 – 3 new cases, and in 2014 – 1 case (ECDC, 2006, 2007, 2008, 2008, 2009, 2010, 2011, 2013, 2014, 2015, 2016). However, it must be remembered that echinococcosis in Latvia is not a compulsory notifiable infectious disease; consequently, the real number of cases may be higher.

Analysing the gender of the patients, the majority of patients – 75 % (87) were female and only 25 % were male, researchers from Poland (Nahorski et al., 2013), China (Zhang et al., 2015, Yu et al., 2008), Czech Republic (Kolarova et al., 2015), Azerbaijan (Vahedi et al., 2012) and Iran (Hajipirloo et al., 2013) have obtained similar data. However, analysing two studies from Peru, one of them emphasised that the majority of patients were male (Moro et al., 2004), but the other – that there was no difference between genders (Reyes

et al., 2012). In a study from Spain (Herrador et al., 2016), it was also concluded that the parasitosis was more common in males. In a study from Italy in different regions, it was concluded that there is no difference between genders (Tamarozzi et al., 2015), but in a study carried out in Sicily, it was concluded that the parasitosis is more common in males (Cappello et al., 2013). Studies from Iran (Sarkari et al., 2010) and Romania (Moldovan et al., 2012) conclude that there is no difference between genders. These differences between genders may be associated with occupation because considering that infection is frequently associated with living conditions, work with cattle and other domestic animals, which will be discussed further, in different countries there are different traditions – whether women or men do those jobs, it could also be important, whether hunting is popular in those regions, which is more common for men, who have closer contact with animals, including dogs, and that supports these gender differences. In economically less developed regions, for example, studies in China, the infection risk may be associated with contaminated water, including work with sewerage systems.

The median age of the patients included in the study upon diagnosis was 54.3, the majority of patients were in the age group of 50 to 60 years, which may be explained by the slow course of the disease because the infection can be detected rarely, but the asymptomatic period is long. Analysing whether the age of either gender differs upon diagnosis, a statistically significant difference was not found. Data from other studies are different and even contradictory. For example, according to the ECDC (ECDC, 2005–2015) data from 2005 to 2010, in most reports mention that the majority of patients in the European region belong to the age group of 45 to 64 years, which are similar to our data. Generally, there are no significant differences in Poland (Nahorski et al., 2013), where one of the studies mentioned that the mean age of patients was 47.7 years. However, there are reports contradictory to our results from other countries, for example, a study from China (Zhang et al., 2015) mentions 45.6

years as the mean age of the patients, a study from Peru, mentions 47 years as the mean age of patients (Moro et al., 2004), a study conducted in Spain concludes that the majority of the patients belong to the age group above 45 years (Herrador et al., 2016); nevertheless, according to other data, the patients belong to the age group of 65–74 years (Rojo-Vasquez et al., 2011). Studies form different regions in Italy conclude that the mean age of patients is 59.8 years and 57 % of patients are older than 60 years (Tamarozzi et al., 2015), but other studies conclude that the mean age is 40 years (Brundu et al., 2014). Examining the Sicily region more accurately, it was concluded that the mean age of patients is 46 years (Cappello et al., 2013). A study from Iran concludes that the highest proportion of patients belong to the age group of 30–39 years (Sarkari et al., 2010) and even 20–39 years (Ahmadi et al., 2011; Gholami et al., 2018), but a study from Pakistan has data that the majority of patients are 20–31 years old (Khan et al., 2018), presumably, the living conditions affect the fact that the patients in those regions are comparatively young. A study from Romania also emphasizes that the highest incidence is in the age group of 45–65 years (Botezatu et al., 2018) and 60–69 years (Moldovan et al., 2012).

In several countries, this disease has been studied among children, for example, in studies from Bulgaria (Jordanova et al., 2015) and Argentina (Dopchiz et al., 2009).

Considering the studies analysed, it can be concluded that in many regions, especially in Europe, there is no significant age difference in patients. Mainly, the patients are of middle age, which could be explained by the fact that the incubation and asymptomatic period is long, which has not made them seek medical attention. Of course, in regions where the disease is very widespread and the exposure to parasite eggs is prolonged and frequent, patients are younger, even children, as mentioned in a study from Bulgaria for example (Jordanova et al., 2015), which in Latvia are only individual cases.

However, it is contradictory with other countries because, for example, in Romania, which is also an endemic region, the age of patients is above 60 years, thus other factors apart from exposure are important presumably, for example, the patients' own or genetic factors, which was also investigated in this study and discussed further.

Analysing the territory of Latvia in context with the residence of patients, it can be concluded that a large number of patients were from Zemgale and from the Northern part of Latgale, whereas practically no cases of this disease are found in Vidzeme. Presumably, it is associated with the number of the disease in the neighbouring countries because in Lithuania there are comparatively many cases, which may explain a higher number of patients in Latgale, while there are comparatively few cases in Estonia (Marcinkute et al., 2013), consequently, the cases in the Northern part of Latvia are fewer. Certainly, the migration of possibly infected animals must be considered.

In this study, complaints that made patients seek medical attention were also analysed. Analysing the complaints, it was concluded that in 73 cases the patients had mentioned that the main complaint was itching skin, which could be explained by more or less pronounced cholestasis that has developed because of compressed bile ducts, but jaundice is not clinically definable yet. In addition, this complaint – itching skin could be associated with an allergic reaction to the parasite. In literature, it is explained by the fact that the parasites are strong producers of IgE (Bakiri et al., 2010); other authors, however, believe that allergisation can be caused by minimal damage from cysts when the content of the cyst comes into contact with the immune system cells (Siracusano et al., 2009). The data of the current study unmistakably confirm the first explanation because it was concluded that some of the patients had elevated alkaline phosphatase levels, which is one of the markers of cholestasis; however, the significance of the second theory cannot be excluded, which should be considered when choosing the methods of treatment.

According to the data from the study, in 29 cases there were complaints about discomfort in the abdomen, which is widely described in literature (Nunnari et al., 2012; Branci et al., 2012, Fischer et al., 2016; Siracusano et al., 2012; Ymeonidis et al., 2013), but in 24 cases excessive fatigue, which is mentioned as an early symptom in literature and could be explained by the interaction of the parasite and the host organism, it is also mentioned that this symptom is observed in alveolar echinococcosis more frequently (Nunnari et al., 2012).

Complaints such as poor appetite, bitterness in the mouth, stomach pain, burning, nausea, vomiting, loose stools, which are also mentioned in publications by other authors and could be associated with hepatomegaly and compression or irritation of the organs that exit the liver (Nunnari et al., 2012; Branci et al., 2012, Fischer et al., 2016; Siracusano et al., 2012; Symeonidis et al., 2013). Usually, the intensity of the complaints is associated with the size of the parasitic tissue, which is characteristic because the parasitic development increases.

Jaundice is also mentioned as one of the initial complaints because of which the patient sought medical attention, but this is associated with the late diagnosis of echinococcosis, when serious complaints have already developed – compression of the main bile ducts or infiltration with the parasitic tissue (Fischer et al., 2016; Siracusano et al., 2012; Ymeonidis et al., 2013; Stojkovic et al., 2015).

It was also concluded that stomach pain was more characteristic for the elderly, which could be associated with a wider spread of the parasitic tissue – wider involvement of the liver and adjacent tissue; whereas excessive fatigue was more characteristic in younger patients, it should be noted that this relationship has not been previously mentioned in the available literature.

The diagnosis was based on two criteria – radiological findings, mainly ultrasound, and serological findings that were according to the recommendations for Europe (WHO, 2003).

It was concluded that the parasitic process had affected the liver in practically all of the patients. However, evaluating the data from the first examination, it can be concluded that diagnosing is difficult because initially, only six patients were diagnosed accurately, thus it is important that the other methods of diagnosis are also used for verification. Most often, a growth in the liver was found after an ultrasound and it was not specified, whereas in 25 cases a growth was simply described as a cyst, which could correspond with the early stages of cystic echinococcosis, when the characteristic features have not developed yet (WHO, 2003). The radiological finding described as tumour was characterised in 18 cases, which could be a differential diagnosis in the cases of alveolar echinococcosis because, as it was mentioned before, the course of this form of echinococcosis is similar to that of malignant tumours (Liu et al., 2014; Kratzer et al., 2015). A lesion in 16 cases was described as haemangioma, which is also described as a differential diagnosis of alveolar echinococcosis (Liu et al., 2014; Kratzer et al., 2015). Rarely, the lesion was described as an abscess or metastasis, as metastasis could be possible differential diagnosis in the case of alveolar echinococcosis (Kratzer et al., 2015); whereas in three cases pathology in the liver was not found in the initial USS, which later proved incorrect, which is difficult to explain. Possible causes could be a small growth or a growth very high in the right lobe of the liver, also the quality of equipment cannot be excluded and is an important criterion.

The initial ultrasound findings were analysed in depth and it was concluded that most frequently the liver is normal in size upon diagnosis, but the number of patients who had surgeries on the liver has a tendency to increase; the right lobe of the liver was involved in the process, which could be explained by the peculiarities of the blood flow in the liver (Nunnari et al.,

2012; Zhang et al., 2015; Agudelo et al., 2016; Piarroux et al., 2011; Branci et al., 2011). The number of patients who have both lobes of the liver involved tends to increase over time, which could also be explained by progression of the disease. As the disease progresses, i.e. over time, the number of patients with 1 focus have a tendency to decrease, which was initially the situation for 61% of patients, but the number of patients with 2 or multiple (≥ 5) foci increases. In other studies, similar data is described (Nunnari et al., 2012; Zhang et al., 2015; Agudelo et al., 2016; Piarroux et al., 2011; Branci et al., 2012; Wuestenberg et al., 2014), for example, in Turkey, 60 % of patients had solitary cysts (Piarroux et al., 2011), in 16.2 % of patients multiple cysts were found, and according to the data from other studies, 20–40 % of patients had multiple cysts (Nunnari et al., 2012). According to the data from a study from Sicily patients had multiple cysts more frequently (60 % of cases) (Cappello et al., 2013).

In addition, the number of patients who had blood-vessels of the liver or adjacent blood-vessels involved tends to increase, the number of patients who had bile ducts involved (infiltrated) tends to increase, which indicates that the therapy used is not effective.

Upon diagnosis, the size of the focus was 6.9 cm (SD ± 4.2), the median was 6.3 cm. According to the data from Turkey, the size of the cysts initially was 5.2 ± 116.5 mm (Wuestenberg et al., 2014), from China – 7.4 cm (Zhang et al., 2015), which are generally similar with the results from the current study. However, a statistically significant relationship that the foci increase over time was not found, considering that, as it will be described further, it was found that the treatment in our opinion did not always conform with the available guidelines.

Given that the number of patients is not high and the disease is progressing comparatively slowly, the question of the best therapy tactic is the source of discussion and in this study, we analysed data on how the patients are

treated and whether a relationship exists between the treatment tactic and the outcome of the disease.

According to the results, it can be concluded that 70.7 % of the patients received the specific therapy with albendazole within one year after diagnosis; however, upon in-depth analysis of the therapy schemes it was observed that in some of the cases therapy was not received completely because within the first year continuous therapy for 6 months was prescribed in only 7.8 % of the cases, but a 6-month course during any period of treatment was received only in 15.5% of the cases. It should be noted that therapy regimens like these cannot be considered optimal because therapy should be received initially for 3 to 6 months and in the cases when the parasitic growth cannot be radically operated it should be regular (Hemphill et al., 2014; Tamarozzi et al., 2014; Vuitton, 2009). In contrast, out of all patients who have not received surgical treatment, only half had received therapy each year. However, it should also be noted that patients who received surgical therapy due to alveolar echinococcosis should receive medication therapy for at least two years (Hemphill et al., 2014; Tamarozzi et al., 2014); nevertheless, in the patients analysed in this study, this relationship was not observed, which increases the risk of relapses of the disease.

In this study, a significant correlation between the type of the parasite and the duration of therapy in one of the parameters was found ($p = 0.028$) – in the cases of *Echinococcus multilocularis* patients had received the therapy for at least 6 months within the first year of treatment, but a further relationship with the total duration of therapy and the number of courses was not observed, which is negative because in inoperable cases therapy has to be continued, possibly for the rest of the life.

Analysing the whole observation period, it was concluded that the mean arithmetic value of treatment was 6.0 (SD ± 8.3) and the median of courses was two, which is very low and it should be noted that some of the patients had

never received any therapy courses, but the highest parameter was 43 courses in the period of observation, and the final data generally shows the weaker spots in our healthcare. In literature, data on similar duration therapy is available (Steinmetz et al., 2014), similar data in a study on the paediatric population (Moroni et al., 2016); whereas the total median of the duration of therapy was 1 year, which is notably low. A statistically significant correlation between how long the patient was observed and how many years they received therapy ($p < 0.001$), and a higher total number of therapy courses ($p < 0.001$) was found.

To assess the effectivity of therapy, two methods were used – changes in the specific antibody titre and changes in the stages of cysts in ultrasounds.

Evaluating the changes in antibody titre, a positive effect was observed in more than a half of patients – they disappeared in 21.6 % of the patients, but the titre decreased in 26.7 % of the patients. A statistically significant correlation between the administration of therapy within one year and the disappearance of antibodies and an ever received 6-month therapy course and disappearance of antibodies. If the duration of therapy and the number of courses increase, a statistically significant probability ($p < 0.001$) that the antibodies will disappear increases.

Evaluating changes in the stage of echinococcosis in context with therapy, a significant relationship between the number of received therapy courses and the median of the stage could be observed – if therapy is received longer and the number therapy courses increases, the minimum of stages is lower, which could indicate that the effect of the treatment is positive, because the activity of the cysts, namely, the parasitic process is reduced. However, the situation is not unique, because similar data on non-conformity to international guidelines is also obtained from other countries (Nabarro et al., 2015).

Analysing the initial stage and its manifestation of the parasitosis at the end of observation, it can be concluded that the increase of stage is 0.2, which

is minimal; however, it can be considered as a positive tendency, it was observed that the stage has a tendency to progress ($p = 0.011$) in treated patients. The above mentioned can be considered as a positive tendency; however, compared to data from the available literature, the results should be more convincing, because, for example, it is mentioned in the available publications that after 1–2 years of therapy 50–75 % 1st stage cysts and 30–55 % 2nd and 3rd stage cysts have become inactive of have disappeared (Nazligul et al., 2015; Stojkoviz et al., 2009).

Unfortunately, other ultrasound parameters – liver size, involvement of liver lobes, number and size of the foci – did not show a relationship with parameters of therapy.

According to the indications in the available literature, IL-4 and IL-10 were evaluated as possible prognostic parameters in this study. A statistically significant correlation with the increase of IL-10 levels and a more severe course of disease in the case of alveolar echinococcosis. Data on IL-10 is also available in the literature – elevated levels could be characteristic of active infection and it has a significance in the survival of the parasite in the cases of cystic (Pang et al., 2014; Mourglia-Ettlin et al., 2011; Shan et al., 2011) and alveolar echinococcosis (Tuxun et al., 2015). In a study on mice, data shows that infected mice had higher IL-10 levels than healthy mice (Pan et al., 2017). In other studies, also done with laboratory animals, it was found that elevated levels of interleukin can indicate the activity and chronisation of the disease (Wang et al., 2014; Hu et al., 2015; Wang et al., 2015).

Studies have also shown that IL-10 levels remain high in patients who are less responsive to surgical or pharmacological treatment (Naik et al., 2016; Amri et al., 2009). Also, data that shows that increased levels of IL-10 are significant, allowing the parasite to avoid the immune system and persist in the host organism is available (Amri et al., 2009). Consequently, we believe that more frequent use of this marker in clinical practice should be considered. The

level of IL-4 in the group of researched patients was below the detection limit; however, it could be explained by the features of echinococcosis because there is data available where in the cases of parasitosis the level of this interleukin could be lower, for example, ≥ 0.39 pg/mL (Petrone et al, 2015). Consequently, in the cases of echinococcosis test systems that have a lower detection limit or other immunological parameters should be used. Also, it is important to note that these immunological markers could serve to improve the diagnostics of echinococcosis; however, they are not specific for this parasitosis; consequently, in order to use those markers, different factors, such as concomitant diseases, age, should be excluded, which is not always possible.

Possible epidemiological risks factors were also evaluated and both similarities and differences with the data from other countries was found.

Of course, it must be noted that, as is to be expected, the selection of trial subjects is very consequential in randomised controlled trials, as it can affect results. Furthermore, the time period between infection by *Echinococcus spp.* eggs and the diagnosis of the disease is typically very long; given that the moment of infection cannot be identified convincingly, identifying risk factors is also difficult.

Using those individuals who did not own dogs and or cats or keep them in controlled conditions, present study provides evidence that coexistence with pet animals that have free access to environment increases the risk of echinococcosis. This evidence also has been proven in previous systematic review and meta-analysis, showing that living in endemic rural areas, in which free roaming dogs have access to offal and being dog owner, seems to be the most highly significant potential risk factors for acquiring particular parasitic infection (Possenti et al. 2016). Dogs allowed to roam or stray dogs have been identified as presenting higher infection risk as they have increased possibilities of finding and ingesting raw carcass meat and offal of fallen livestock. In contrast, dogs that cannot roam freely, like household pets, commonly present

lower infection rates, which may be due to a diet comprising mainly of cooked food or kitchen scraps that are unlikely to contain viable hydatid cysts. However, such differences in relative infection rates may also be explained by the fact that dogs which are allowed to roam free are less likely to receive regular anthelmintic treatment (Campos-Bueno et al. 2000). However, present study showed that the individuals who were keeping dogs in kennels or lashes near dwellings has significantly higher risk of CE infection.

From the present results it can be deduced that the risk of echinococcosis was strongly associated were closely related to living in rural dwelling and with being a farmer of livestock animals. The potential risk factors as "living in rural areas", "slaughter at home" and "slaughterhouse" were shown to have significantly higher odds of infection (Possenti et al. 2016). Living in rural dwelling and being a farmer or rancher may increase the duration of exposure to risk factors and, thus, increase possibility of being infected with echinococcus eggs.

Present study indicates that there is a greater risk of echinococcosis for individuals who have hunters in family. Hunters seem to be an under examined population, which has not been studied in Latvia so far. This social group is heterogeneous in terms of social provenance, experience in hunting, and average time spent in the forest environment. The research conducted among Austrian hunters has shown 5 and 11 % seropositivity for *E. multilocularis* and *E. granulosus*, respectively (Deutz et al. 2003). Zukiewicz-Sobczak et al. (2014) estimated seropositivity for *E. granulosus* among Polish foresters at 3.2 %. Hunters, who spend time in forested and wild areas, may have contact with many causative agents of zoonoses through exposure to ticks, dead rodents, and birds, as well as excreta/secretions of wild animals or contaminated food, water, and soil (Richard and Oppliger 2015).

For the remaining potential risk factors such as “having a kitchen garden”, “gathering mushrooms or berries”, and “eating unproperly cooked meat” posed no excess risk of disease.

One of the aims and tasks of the study was to identify the MHC HLA Class II genes found in echinococcosis patients and their relevance to disease severity.

One of the objectives and tasks of the study was to determine HLA Class II alleles occurring among echinococcosis patients and their association with the severity of the disease.

It must be mentioned that literature data on the development of echinococcosis and immunogenetic factors of patients are limited and in the PubMed database only 10 publications could be used, because a certain number of studies were not available in English or Russian, as well as, for some studies, the original publications themselves were not available.

The results showed that of HLA DRB1 gene alleles *17:01 and *07:01 are more frequently found in patients with cystic echinococcosis. The association of allele *07:01 with increased risk of the disease has been described in China; however, it must be added that the Chinese publication refers to alveolar echinococcosis (Mosayebi et al., 2013). Alleles *01:01 and *15:01 rarely occur among the patients in the cystic echinococcosis group. Similar data on the protective action of allele *01:01 are mentioned in studies from Russia (Mosayebi et al., 2013), Lebanon (Aydinli et al., 2007), Saudi Arabia (Chakhtoura et al., 2007) and Turkey (Yalcin et al., 2010). Alleles *17:01, *11:01 and *07:01 occur more frequently in patients with alveolar echinococcosis. It must be mentioned that contradicting data are available regarding allele *11:01 in a study conducted in Germany, where this allele is considered to be protective in the cases of alveolar echinococcosis (Eiermann et al., 1998), similar data have been obtained in Turkey (Yalcin et al., 2010); meanwhile the studies conducted in different European countries suggest that it

has a protective character in the event of cystic echinococcosis (Lukmanova et al., 2011). Allele *01:01 was identified as protective in the event of alveolar echinococcosis. It must be added that data similar to our data have been obtained from the nearest regions, for instance, Europe or Russia, while partially differing data come from different Asian countries and these differences are, potentially, due to genetic differences in race. Association with allele combinations was also anaysed and it was concluded that in all patients *04:01/*11:01, *17:01/*13:01 and *11:01/*13:01 were connected to more severe course of the disease. It was *04:01/*11:01 in cystic echinococcosis group and *17:01/*13:01 in alveolar echinococcosis group. No protective combinations were found. There is no significant data about allele combination in the literature (author's comment).

Analysis of HLA-DQB1 alleles leads one to the conclusion that alleles *03:02 and *03:01 are credibly more frequent among cystic echinococcosis patients, while allele *05:01 prevails in the alveolar echinococcosis patient group. Here our data contradict with literature data from Iran, where this allele is directly associated with a lower risk of cystic echinococcosis development (Mosayebi et al., 2013), which could be explained by the fact that the population of this region in itself could have significant genetic differences from the Latvian population. Also alleles *05:01 and *06:02-8 had protective effect in cystic echinococcosis group but they might pose higher risk in alveolar echinococcosis group. Regarding allele combinations *03:01/*03:02, *02:01-2/*03:01 and *03:02/*06:02 were connected with more severe disease in all patients but *03:01/*06:02-8 might be protective. In cystic echinococcosis patient group *02:01-2/*03:01, *03:01/*03:02 and *03:02/*06:02-8 were connected with more severe disease and it was only one in alveolar echinococcosis group – *03:01/*03:02.

Analysis of HLA-DQA1 alleles leads one to the conclusion that allele *04:01, is credibly more frequent among cystic echinococcosis patients, while

allele *01:02 occurs rarely. Finding of statistically significant alleles in the group of alveolar echinococcosis was not successful. The available literature lacked information on the association of alleles HLA-DQA1 with echinococcosis. The data obtained could be used in similar studies in other Baltic states, as well as European Union countries, which could expand the data base used for the detection of similarities and differences. Allele combinations *01:03/05:01 and *03:01/05:01 were connected with more severe course of diasese in all patients and *01:01/02:01 in alveolar echinococcosis group.

In-depth analysis brings one to the conclusion that the following haplotypes can be identified in the cystic echinococcosis group with a more severe course of disease: HLA-DRB1*04:01/DQB1*03:01/DQA1*03:01, HLA-DRB1*11:01/DQB1*03:01/DQA1*05:03. Meanwhile the following genotypes can be identified in the alveolar echinococcosis group: HLA-DRB1*17:01/DQB1*02:01-2/DQA1*01:01 and HLA-DRB1*11:01/ DQB1*03:01/DQA1*03:01

The following haplotypes can be identified as protective in all patient groups: DRB1*15:01/DQB1*06:02-8/DQA1*03:01 and HLA-DRB1*13:01/ DQB1*02:01-2/DQA1*05:01.

In this study, possible risk factors of behaviour and genetics were studied; however, the author notes that they are not the only significant factors. In the development of the disease, factors of the parasite itself are significant, for example, genotype, which has not been investigated in this study, but obtaining the data in other prospective studies should be considered.

Immunogenetic data could be significant for therapy planning according to the peculiarities of the patient because the data on the optimal length of therapy and whether it is possible to discontinue it without furthering the relapse of the infection is not available at present. It is known that there are patients with slow progression of the disease and without serious complications, but it should be remembered that there is also a group of

patients who develop a rapid increase in activity of the parasitic process if therapy is discontinued.

In this study, it was concluded that 31 out of 116 patients died, 13 of which the cause of death was echinococcosis, the death of 5 patients may be associated with echinococcosis, as the cause of death was associated with liver pathology. However, it should be considered that this number could be higher because it is possible that the effect of echinococcosis on the organism in general has not been evaluated, and, for example, in the cases where the cause of death was infection, it is possible that echinococcosis had been a significant contributor of death, considering the above-mentioned changes in liver parameters, which could have caused disorders in protein synthesis, including antibodies, or the mentioned interleukin changes, which could have worsened the course of infection.

As a result of this study, it can be concluded that the condition of the patient, characterised by biochemical and immunogenetic parameters, has a significant role in the effectiveness of therapy, and, considering them, it must be taken into account that it is possible to improve the effectiveness of therapy. Results also confirm that immunogenetic factors can give an important insight about the course of the disease, including the activity of the parasitic process.

The conducted study provides important information – genetic predisposition and possible defence of the immune system against the development of echinococcosis. It is necessary to continue studying the molecular mechanism, in order to forecast the outcome of the disease, which would mean that new ways and approaches of treatment as well as prepared therapy options are offered.

4. CONCLUSIONS

1. In Latvia, statistically significant risk factors for echinococcosis were:
    1) living in a rural household, particularly for a long time;
    2) dogs and cats have been owned;
    3) dogs and cats live unattended and have eaten small rodents or internal organs of slaughtered animals;
    4) the fact that they owned livestock;
    5) the fact that there are hunters in family.
2. There is higher probability that antibodies will disappear as a result of treatment if:
    1) therapy has been started 1 year after establishing diagnosis;
    2) patient had 6-month course of treatment;
    3) therapy has been every year;
    4) with increasing treatment duration and number of courses, the likelihood of antibody disappearance increases.
3. There is a correlation between the cystic echinococcosis stage and the patients receiving therapy.
4. Increases in IL-10 levels can be used as a sign of a more severe and complicated course of disease in alveolar echinococcosis.
5. The risk of dying is statistically significantly increased by elevated levels of ESR, SF, GGT and bilirubin.
6. In case of cystic echinococcosis more severe course of disease is connected to HLA -DRB1 alleles *17:01 and *07:01, DQB1*03:01 and 03:02, DQA1*04:01 and haplotypes HLA-DRB1*04:01/DQB1*03:01/ DQA1*03:01, HLA-DRB1*11:01/DQB1*03:01 /DQA1*05:01.

7. In case of alveolar echinococcosis more severe course of disease is connected to HLA DRB alleles *17:01 and *07:01, DQB1*05:01 and haplotypes HLA-DRB1*17:01/DQB1*02:01-2/DQA1*01:01, HLA-DRB1*11:01/QB1*03:01/QA1*01:03 and HLA-DRB1*11:01/ DQB1*03:01/DQA1*03:01.
8. In case of cystic echinococcosis HLA DRB1 alleles *15:01, DQB1*05:01 and *06:02-8, DQA1 *01:02 and in case of alveolar echinococcosis HLA DRB alleles *01:01, *15:01 and *13:01, HLA DQB1*06:02-8, HLA DQA1*01:02 and *05:01 are protective.
9. Protective haplotypes in all echinococcosis patients are DRB1*15:01/ DQB1*06:02-8/DQA1*03:01 and HLA-DRB1*13:01/DQB1*02:01-2/ DQA1*05:01.

5. PRACTICAL RECOMMENDATIONS

1. Diagnostic recommendations:
    1) For diagnosis of echinococcosis based on our results and case definition, it is recommended to use both radiological and serological findings. A convincing radiological finding for diagnosis must be valued higher than a negative serological finding.
    2) To diagnose echinococcosis, use all of the following: ELISA, IF, IB, serological diagnostic methods, taking into account that the first two are more sensitive but the third one more specific. It should also be noted that one negative result does not exclude the diagnosis and it is advised to use them all.
2. Treatment recommendations:
    1) Medical treatment:
        - must be started as soon as possible after diagnosis;
        - must be at least 6 months long, followed by USG data evaluation;
        - should be long enough if radical resection of the parasite is not possible; then antiparasitic drugs should be used life-long.
    2) Planning the duration of the treatment, the identification of genetic alleles could be used in uncertain cases because it has been proved that there are individual alleles that could define a more complicated course of the disease.
    3) The results of the treatment should be evaluated in complexity, using radiological data, serological and immunological data and we must not rely on only one parameter.

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PUBLICATIONS AND PRESENTATIONS

Published articles (4)

1. **Laivacuma, S.**, Deksne, G., Jokelainen, P., Ivanovs, A., Zaharova, L., Zeltiņa, I., Vīksna, L., Krūmiņa, A. Risk Factors for Human Cystic Echinococcosis in Latvia. *Vector Borne Zoonotic Dis*. 2019 Feb 25. doi: 10.1089/vbz.2018.2354. [Epub ahead of print] PubMed PMID: 30801230.
2. **Laivacuma, S.**, Eglīte, J., Derovs, A., Vīksna, L. Distribution of HLA Allele Frequencies in Patients with Cystic and Alveolar Echinococcosis in Latvia. *Proc. Latv. Acad. Sci. Sect. B nat. Exact appl. Sci*. 2019; 73(4), pp. 296-303.
3. **Laivacuma, S.**, Ivanovs, A., Derovs, A., Viksna, L. Cystic echinococcosis: epidemilogical and clinical aspects of Latvian population and review of the literature. *Eksp Klin Gastroenterol*. 2015; (7): 24–30. Review. PubMed PMID: 26817119.
4. Marcinkutė, A., Šarkūnas, M., Moks, E., Saarma, U., Jokelainen, P., Bagrade, G., **Laivacuma, S.**, Strupas, K., Sokolovas, V., Deplazes, P. Echinococcus infections in the Baltic region. *Vet Parasitol*. 2015 Oct 30; 213 (3–4): 121-31. doi: 10.1016/j.vetpar.2015.07.032. Epub 2015 Jul 31. Review. PubMed PMID: 26324242.

Abstracts and participation in international congresses and conferences (5)

1. **Laivacuma, S.**, Zeltina, I. Echinococcus multilocularis infection of the liver: a clinical case. Collection of Scientific Papers 2017: Research articles in medicine & pharmacy: Abstracts from VIII Latvian Gastroenterology

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2. **Laivacuma, S**., Eglīte, J., Vīksna, L. Risk factors of echinococcosis in Latvia//7th Conference of the Scandinavian-Baltic Society for Parasitology (Riga, Latvia, June 8-9, 2017): [Abstracts]. – Riga, 2017. p.27.
3. **Laivacuma, S.,** Eglite, J., Derovs, A., Viksna, L. Characteristics and HLA II class polymorphism in patients with alveolar echinococcosis in Latvia// Collection of Scientific Papers 2015: Research articles in medicine & pharmacy: Abstracts from VII Latvian Gastroenterology Congress with International participation [Riga, Latvia, Dec.5, 2015]/Rīga Stradiņš University. – Rīga: RSU, 2016. Suppl.1, p.87.
4. **Laivacuma, S.,** Krumina, A., Viksna, L. Pelvic bone and hip joint hydatid disease misdiagnosed as tuberculosis: a clinical case//Symposium "Innovation for the management of echinococcosis" (Besançon, France, Mar.27-29, 2014): [Abstracts]. - Besançon, 2014. p.154.
5. **Laivacuma, S.** Liver biochemical parameters in patients with liver echinococcosis//Collection of Scientific Papers 2013: Research articles in medicine & pharmacy: Abstracts from VI Latvian Gastroenterology Congress with International participation (Riga, Latvia, Dec.7, 2013)/Riga Stradiņš University. – Riga, 2013. Suppl.1, p. 36.

Abstracts and participation in local congresses and conferences (6)

1. **Laivacuma, S.**, Viksna, L. Specifisko antivielu titra izmaiņas kā ehinokokozes terapijas efektivitātes kritērijs. 2018. gada Zinātniskās konferences tēzes (Rīga, 2018.g. 22.-23.martā)/Rīgas Stradiņa universitāte. – Rīga, 2018. 12.lpp

2. **Laivacuma, S**., Vīksna, L. Ehinokokozes slimnieku viedoklis par savu slimību// 2015.gada Zinātniskās konferences tēzes (Rīga, 2015.g. 26.-27.martā)/Rīgas Stradiņa universitāte. – Rīga, 2015. 193. lpp.
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4. **Laivacuma, S.,** Vīksna, L. Alveolārās ehinokokozes slimnieku analīze//2014.gada Zinātniskās konferences tēzes (Rīga, 2014.g. 10.-11.aprīlī) / Rīgas Stradiņa universitāte. – Rīga, 2014. 184. lpp.
5. **Laivacuma, S.,** Vīksna, L. Ehinokokozes medikamentozās ārstēšanas rezultāti Latvijā//2013.gada Zinātniskās konferences tēzes (Rīga, 2013.g. 21.-22.martā)/Rīgas Stradiņa universitāte. – Rīga, 2013. 195. lpp.
6. Vīksna, L., Aldiņš, P., Zeltiņa, I., Vilmanis, J., **Laivacuma, S.** Ehinokokozes diagnostikas un ārstēšanas taktika//2010.gada Zinātniskās konferences tēzes (Rīga, 2010.g. 18.-19.martā)/Rīgas Stradiņa universitāte. – Rīga, 2010. 194. lpp.