

Experimental Study Upon the Virulence of Infectious Microbial Agents Involved in Violent Deaths Presenting Septic States

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The study of bacterial pathogeny represents a very important issue for the forensic specialists, clinicians, managers and healthcare policymakers. The purpose of this experimental research is to study the virulence of some infectious microbial agents (Pseudomonas Aeruginosa, Staphylococcus Aureus, Escherichia Coli, Proteus Mirabilis, Erysipelothrix Rhusiopathiae), some of them frequently involved in violent deaths presenting septic states, by inoculating them in white mice Mus Musculus (MMs), in the presence of competing factors of death, reproducing this way a model of human violent death consecutive of septic complications that occur in the evolution of some traumas. The results of the study show that the survival period of the MMs depends on the microbial agent of the inoculated strain, the dilution of the culture suspension, the presence of associated lesions, age. In decreasing order, according to the value of LD₅₀, the most virulent strains are Erysipelothrix Rhusiopathiae, Escherichia Coli, Proteus Mirabilis, followed by Staphylococcus Aureus and Pseudomonas Aeruginosa. The lethality index increases proportionally with the virulence of the etiologic agent and the presence of the associated lesions, confirming once again the similarity with physiopathological mechanisms that exist in human pathology.

Keywords: sepsis, violent death, infectious microbial agents, lethal dose 50, mouse

The existence in the medico-legal records of violent deaths from septic cause, the indirect causality that leads to their occurrence in the evolution of physio pathological mechanism of different diseases, the juridical implication of medical fault regarding nosocomial infection and medical practice, the high rate of mortality of severe sepsis and septic shock in ICU and hospitals and high use of resources, all these make the study of bacterial pathogeny an issue of big concern, both for the forensic specialist and also for clinicians, managers and healthcare policymakers [1-4]. In these cases, medico-legal autopsy is extremely important to establish the cause of death of the patient [5-6]. Sepsis represents a major public health concern; it should be defined as *life-threatening organ dysfunction caused by a dysregulated host response to infection* [7]. Organ dysfunction is represented by an increase in the Sequential [Sepsis-related] Organ Failure Assessment (SOFA) score of 2 points or more, which is associated with an in-hospital mortality greater than 10% [7]. Septic shock should be defined as a subset of sepsis in which particularly profound circulatory, cellular, and metabolic abnormalities are associated with a greater risk of mortality than with sepsis alone [7,8].

Factors significantly associated in sepsis with increased risk of death are: older age, male sex, immune deficiency/pre-existing immunosuppression (patients with cancer, cirrhosis, etc.), admission hyperglycaemia [1, 9-12]. Advances in early management of septic shock patients have improved survival at the initial phase, but the risk of death persists in the medium term, in the first 3 months. In a study conducted by Pavon et al. (2013), at 3 months, 52.2% of patients had died [1]. Sepsis is considered to induce immune suppression (reduced expression of genes

involved in gluconeogenesis and glycolysis) leading to increased susceptibility to secondary infections with associated late mortality [13].

The following biomarkers may be used to help diagnose patients with sepsis: PCT (procalcitonin), CRP (C-reactive protein), Pro-ANP (atrial natriuretic peptide), Adrenomedullin, IL-6 (interleukin), Selenium, TIMP-1 (tissue inhibitor of metalloprotease). Physiologic parameters more useful for the diagnosis of sepsis are: PMNs (polymorphonuclear cells), WBCs (white blood cells), temperature, Leukocyte count, PT (prothrombin time), INR (international normalized ratio), APTT (activated partial thromboplastin time), Fibrinogen, Platelet counts, Bleeding time, Fibrinopeptide A, TAT (thrombin-antithrombin complex), Fragment 1.2, FDPs (fibrin degradation products), D-dimer [2]. In patients with severe sepsis, progressive hypocoagulability is associated with increased risk of death and increased risk of bleeding [14]. In order to demonstrate bacterial pathogeny numerous techniques were elaborated, both in vitro and in vivo [15] and they offer much more accurate indicators about germs pathogeny, being done by qualitative/quantitative inoculation tests [16,17-19].

The purpose of this experimental study is to determine the virulence of some infectious microbial agents (*Pseudomonas Aeruginosa*, *Staphylococcus Aureus*, *Escherichia Coli*, *Proteus Mirabilis*, *Erysipelothrix Rhusiopathiae*), some of them frequently involved in violent deaths presenting septic states, by inoculating them in white mice *Mus Musculus* (MMs), in the presence of competing factors of death, reproducing this way a model of human violent death consecutive of septic complications that occur in the evolution of some traumas.

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Experimental part

Materials and methods

The study was conducted in the Legal Medicine Bihor County Service and Faculty of Medicine and Pharmacy of Oradea, between February 2012 - June 2013. The research was performed in compliance with good laboratory practice, in accordance with the European Convention principles for the protection of vertebrate animals used in experimental and other scientific purposes, adopted in 1986, in Strasbourg [20], the 2010/63/EU Directive of the European Parliament and of the European Council, adopted on 22 September 2010 [21] on the protection of animals used for scientific purposes. It was also in accordance with the Romanian law for animal experimentation and it had the acceptance of the Ethics Commission of the Council of the Faculty of Medicine and Pharmacy, University of Oradea.

The experimental study was conducted on a group of 120 MMs, coming from the bio base laboratory of Faculty of Medicine and Pharmacy of Oradea. MMs included in the study were adults or young, of both sexes, with ages between 3 weeks and 2 months, with weight of 10-15g (young ones) and 18-25 g (adults). The study was performed by inoculating to MMs some pathogen agents as strains tested on microbial cultures (in liquid environment or suspension from the agar culture) and suspensions obtained directly from pathological human products. For this purpose, in the first phase of the study, the pathogen agents were identified in different biological samples harvested from cadaver, on the occasion of medico-legal autopsy, in the Legal Medicine Bihor County Service. The assumption was that there is a correspondence between ante mortem bacterial flora and the one possible identified post-mortem. The selected medico-legal cases were represented by the violent ones where in the evolution of trauma appeared septic complications. The existence of a positive bacteriologic diagnosis ante mortem for these pathogen agents excludes the possibility of cadaver contamination during the transport, disposal or autopsy manoeuvres.

From the microbial agents, were selected the species more frequently encountered: *Bacillus Pyocyaneus* (*Pseudomonas Aeruginosa*) (gram-negative bacillus), *Staphylococcus Aureus* (gram-positive cocci), *Escherichia Coli* (gram-negative bacillus) and *Bacillus Proteus Mirabilis* (gram-negative bacillus), also mentioned in the specialty literature [22-24]. The experiment was completed by choosing infectious agents with species tropism that is *Erysipelothrix Rhusiopathiae* (*Bacillus Rujet*) (nonsporulating, nonencapsulated gram-positive rod-shaped aerobic or facultative anaerobic organism), microbial agent that produces a high number of lethal sepsis infections in MMs [25, 26].

The harvesting of pathological products (blood, urine, tracheal secretion, secretion from different wounds, fragments of pulmonary tissues) from the human cadaver was done in the first 1-4 hours from death in concordance to the general norms of asepsis, sampling, conservation and inoculation. The route of administration of the microbial agent was intraperitoneal inoculation, this being considered one of the traumatic factors incriminated in the mechanism of death. Prior to the inoculation procedure, in half of the total number of MMs from the study group it was produced an associated lesion (a small burn of the skin, at the level of the inoculation place, with the help of flame heated rod) as a competing factor in the mechanism of death. The action of the traumatic agent of small to median intensity is considered as being an associated

factor with implications in the physio pathological complex mechanism that appear in the occurrence of the death.

The experimental study consisted in the determination of acute lethal dose 50 (LD_{50}) for every inoculated pathogen: *Bacillus Pyocyaneus* (*Pseudomonas aeruginosa*), *Staphylococcus aureus*, *Escherichia coli*, *Proteus Mirabilis*, *Erysipelothrix Rhusiopathiae*. The LD_{50} is a statistically derived amount of a substance that can be expected to cause death in 50% of the animals when given by a specified route as a single dose and the animals observed for a specified period of time [27, 28]. The following principles were respected: the minimum number of MMs must be 5; the critical dilution 50% must be framed by at least two superior dilutions and two inferior dilutions [25, 26, 29]. The 120 MMs were divided in groups of 6, of both genders, young and adults, some of them with previous traumatic lesions, other without (in equal proportions). The results were registered in tables, where it was noted the number of dead/survivor MMs at every administered dilution.

For the determination of LD_{50} , the method of cumulative totals was applied, and for every dilution it was calculated the percentage of morbidity. The correction coefficient was determined χ^2 (chi squared), for measuring the difference between the variation series obtained after inoculation with the pathological agent of corresponding DL_{50} , the value of χ^2 being obtained on the basis of the quadruple table. Also, it was determined the relative index F regarding the deaths in the studied groups and the lethality coefficient L [30, 31]. At the end of the experimental phase, all MMs were autopsied, the survivor ones after a previous narcosis with ether. During the autopsy biological samples were harvested (blood, tissue fragments) to effectuate the microbiological and histopathological exam.

Results and discussions

The results obtained after inoculation with different species of pathogenic agents are presented in table 1. For the determination of LD_{50} the method of cumulative total was applied, the results were registered in columns 6-9.

The formulas for the calculation of the correcting factors, respectively for χ^2 (chi squared) are: *Correction coefficient* = (% of mortality at the dilution immediately superior to the one of 50% - 50) / (% of mortality at the dilution immediately superior to the one at 50% - % of mortality at the dilution immediately inferior to the one of 50%)

$$\chi^2 = \left[\frac{(a_1 \times n_6 - b_1 \times n_5)^2}{a_1 + b_1} + \frac{(a_2 \times n_6 - b_2 \times n_5)^2}{a_2 + b_2} + \frac{(a_3 \times n_6 - b_3 \times n_5)^2}{a_3 + b_3} + \frac{(a_4 \times n_6 - b_4 \times n_5)^2}{a_4 + b_4} \right] \times \frac{1}{n_5 \times n_6}$$

In table 2 are presented the values used for calculation of χ^2 .

The parameters calculated for different species of pathogenic agent are mentioned in table 3, and table 4 gives LD_{50} depending on the selected MMs groups.

Table 5 presents the evolution of the deaths as a consequence of the inoculation of the pathogenic agent at the dilution of chosen LD_{50} , depending on the presence or absence of associated lesions.

Concluding, the calculation of dilutions regarding each culture of inoculated pathogenic agent (fig. 1 referring to the case of critical dilutions), revealed that the most virulent strains are in descending order *Erysipelothrix Rhusiopathiae*, *E. Coli* and *B. Proteus mirabilis*. These were reflected by

Table 1
CALCULATION OF DL₅₀ IN MMs INOCULATED WITH DIFFERENT PATHOGENIC AGENTS

Dilution of Suspension	Number MMs				Cumulative totals			
	Inoculated	Dead	Survivors	Mortality rate	Dead	Survivors	Mortality rate	%
1	2	3	4	5	6	7	8	9
<i>Bacillus Pyocyaneus (Pseudomonas Aeruginosa)</i>								
10 ⁻¹	5	5	0	5/5	9	0	9/9	100
10 ⁻²	5	3	2	3/5	4	2	4/6	66.7
10 ⁻³	5	1	4	1/5	1	6	1/7	14.3
10 ⁻⁴	5	0	5	0/5	0	11	0/11	0
<i>Bacillus Proteus Mirabilis</i>								
10 ⁻¹	5	5	0	5/5	16	0	16/16	100
10 ⁻²	5	5	0	5/5	11	0	11/11	100
10 ⁻³	5	3	2	3/5	6	2	6/8	75
10 ⁻⁴	5	2	3	2/5	3	5	3/8	37.5
10 ⁻⁵	5	1	4	1/5	1	9	1/10	10
10 ⁻⁶	5	0	5	0/5	0	14	0/14	0
<i>Escherichia Coli</i>								
10 ⁻¹	5	5	0	5/5	17	0	17/17	100
10 ⁻²	5	4	1	4/5	12	1	12/13	92.3
10 ⁻³	5	4	1	4/5	8	2	8/10	80
10 ⁻⁴	5	3	2	3/5	4	4	4/8	50
10 ⁻⁵	5	1	4	1/5	1	8	1/9	11.1
10 ⁻⁶	5	0	5	0/5	0	13	0/13	0
<i>Staphylococcus Aureus</i>								
10 ⁻¹	5	5	0	5/5	10	0	10/10	100
10 ⁻²	5	4	1	4/5	5	1	5/6	83.3
10 ⁻³	5	1	4	1/5	1	5	1/6	16.7
10 ⁻⁴	5	0	5	0/5	0	10	0/10	0
<i>Bacillus Rujet (Erysipelotrix Rhusiopathiae)</i>								
10 ⁻¹	5	5	0	5/5	20	0	20/20	100
10 ⁻²	5	5	0	5/5	15	0	15/15	100
10 ⁻³	5	4	1	4/5	10	1	10/11	90.9
10 ⁻⁴	5	3	2	3/5	6	3	6/9	66.7
10 ⁻⁵	5	2	3	2/5	3	6	3/9	33.3
10 ⁻⁶	5	1	4	1/5	1	10	1/11	9.1
10 ⁻⁷	5	0	5	0/5	0	15	0/15	0

Table 2
DETERMINATION OF CRITICAL DILUTION

MMs	Deceased	Survivors	Total
Young without lesions	(a1)	(b1)	6(n1=a1+b1)
Young with lesions	(a2)	(b2)	6(n2=a2+b2)
Adults without lesions	(a3)	(b3)	6(n3=a3+b3)
Adults with lesions	(a4)	(b4)	6(n4=a4+b4)
Total	(n5)	(n6)	24(N)

Table 3
CALCULATED PARAMETERS FOR DIFFERENT SPECIES OF PATHOGENIC AGENTS

Pathogenic agent	Correction Coefficient	logDL ₅₀	χ ²
<i>Bacillus Pyocyaneus (Pseudomonas Aeruginosa)</i>	0.32	log10 ² +(log10x0.32)=2.32	3.428
<i>Bacillus Proteus Mirabilis</i>	0.67	log10 ² +(log10 x0.67)=3.67	3.428
<i>Escherichia Coli</i>	0.42	log10 ² +(log10x0.42)=4	4.5
<i>Staphylococcus Aureus</i>	0.5	log10 ² +(log10x0.5)=2.5	3.188
<i>Erysipelotrix Rhusiopathiae</i>	0.5	log10 ² +(log10x0.5)=4.5	5.445

MMs characteristics	Deceased	Survivors	Total
<i>Bacillus Pyocyaneus (Pseudomonas Aeruginosa)</i>			
Young without lesions	3(a1)	3(b1)	6(n1=a1+b1)
Young with lesions	4(a2)	2(b2)	6(n2=a2+b2)
Adults without lesions	1(a3)	5(b3)	6(n3=a3+b3)
Adults with lesions	2(a4)	4(b4)	6(n4=a4+b4)
Total	10(n5)	14(n6)	24(N)
<i>Bacillus Proteus Mirabilis</i>			
Young without lesions	3(a1)	3(b1)	6(n1=a1+b1)
Young with lesions	5(a2)	1(b2)	6(n2=a2+b2)
Adults without lesions	2(a3)	4(b3)	6(n3=a3+b3)
Adults with lesions	4(a4)	2(b4)	6(n4=a4+b4)
Total	14(n5)	10(n6)	24(N)
<i>Escherichia Coli</i>			
Young without lesions	3(a1)	3(b1)	6(n1=a1+b1)
Young with lesions	6(a2)	0(b2)	6(n2=a2+b2)
Adults without lesions	3(a3)	3(b3)	6(n3=a3+b3)
Adults with lesions	4(a4)	2(b4)	6(n4=a4+b4)
Total	16(n5)	8(n6)	24(N)
<i>Staphylococcus Aureus</i>			
Young without lesions	3(a1)	3(b1)	6(n1=a1+b1)
Young with lesions	5(a2)	1(b2)	6(n2=a2+b2)
Adults without lesions	2(a3)	4(b3)	6(n3=a3+b3)
Adults with lesions	3(a4)	3(b4)	6(n4=a4+b4)
Total	13(n5)	11(n6)	24(N)
<i>Erysipelotrix Rhusiopathiae</i>			
Young without lesions	3(a1)	3(b1)	6(n1=a1+b1)
Young with lesions	6(a2)	0(b2)	6(n2=a2+b2)
Adults without lesions	3(a3)	3(b3)	6(n3=a3+b3)
Adults with lesions	5(a4)	1(b4)	6(n4=a4+b4)
Total	17(n5)	17(n6)	24(N)

Table 4
LD₅₀ DEPENDING ON THE GROUPS OF MMs
SELECTED FOR DIFFERENT SPECIES OF
PATHOGENIC AGENTS

Pathogenic agent	MMs %			
	Young		Adults	
	Without lesions	With lesions	Without lesions	With lesions
<i>Bacillus Pyocyaneus</i>	50	60.67	16.67	33.33
<i>Bacillus Proteus Mirabilis</i>	50	83.33	33.33	66.67
<i>Escherichia Coli</i>	50	100	50	66.67
<i>Staphylococcus Aureus</i>	50	83.33	33.33	50
<i>Erysipelotrix Rhusiopathiae</i>	50	100	50	83.33

Table 5
EVOLUTION OF DEATHS AS A CONSEQUENCE
OF THE INOCULATION OF THE PATHOGENIC
AGENTS AT THE CHOSEN LD₅₀, DEPENDING ON
THE PRESENCE OR ABSENCE OF ASSOCIATED
LESIONS

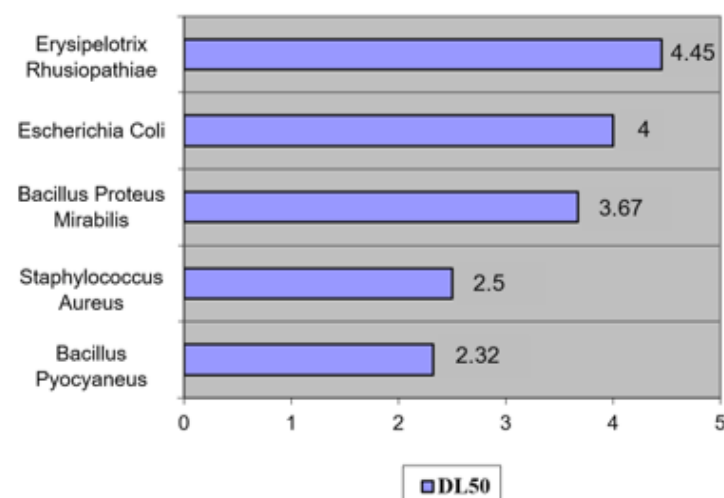


Fig. 1. LD₅₀ of inoculated infectious agents in the young
MM groups

the increased number of deaths with preponderance in the MMs groups with previous associated lesions.

Calculation of the relative indexes concerning the deaths in the studied MMs groups

As well, it was calculated the values of the relative indexes concerning the number of deaths reported to the

total number of cases for each group. Using the calculation formula of the relative indexes, the following values (table 6) were obtained. For a better understanding, an exemple (by selection of the MMs group inoculated with *B. Pyocyaneus* and choosing the groups of adults without lesions) is given:

$$F = \frac{n}{N}$$

$$F = \frac{1}{6} = 0.17$$

where n - the number of deaths for each group, N - number of total studied cases for each selected group. By analyzing the entire group of MMs, the relative indexes of deaths were obtained (table 6).

In young MMs without lesions, the values of F are all 0.5 because it was considered for inoculation the critical dilution of the suspension culture, which produces death in 50% of them. Extending the study to all groups of mice, the relative indexes were calculated (choosing as criteria of selectivity the group young/adults and with/without lesions). The values in the table represent relative indexes, meaning the number of deaths reported to the total number of the cases included in each group. In the column with/without lesions, it was considered for calculation the sum of young MMs, but also adult mice. Similarly, in the column young/adults it was considered the sum of MMs with and without lesions. It is exemplified below, by choosing the group of MMs with *B. Pyocyaneus*, the group of adults without lesions. Using the formula of calculating the relative frequencies, it resulted:

$$F = \frac{3 + 1}{6 + 6} = 33.3\%$$

Extrapolating the calculation to the groups of MMs, the results depicted in table 7 were obtained. It is also graphically showed as cumulative, in figure 2.

Calculation of lethality coefficient L

Another indicator in the case of the MMs death is the lethality coefficient (or the proportional mortality) or the lethality index (L). This represents the death proportion of a certain category from the total deaths, permitting to establish the grades of mortality and show the frequency of deaths determined by an etiology agent compared to all deaths. It was calculated the lethality index for every studied category (with or without lesions, young or adults). The results are presented in columns 6-9 from table 8. For exemplification, the lethality index for *B. Rujet* for the adult MMs was calculated bellow:

$$L = \frac{D_c}{D} \times 100 = \frac{8}{30} \times 100 = 26.7$$

where: L - lethality, D - numbers of deaths of a certain cause (columns 2-5); \bar{D} - total number of MMs deceased. The lethality index increases directly with the virulence of the etiologic agents and with the presence of associated lesions, confirming once again the similitude with the

Pathogenic agents	MMs groups			
	Young		Adults	
	Without lesions	With lesions	Without lesions	With lesions
<i>Bacillus Pyocyaneus</i>	0.50	0.67	0.17	0.33
<i>Bacillus Proteus Mirabilis</i>		0.83	0.33	0.50
<i>Escherichia Coli</i>		0.83	0.33	0.67
<i>Staphylococcus Aureus</i>		1.00	0.50	0.67
<i>Erysipelotrix Rhusiopathiae</i>		1.00	0.50	0.83

Table 6
RELATIVE INDEXES OF DEATHS

Pathogenic agents	Groups of MMs (% deaths)			
	Without lesions	With lesions	Without lesions	With lesions
<i>Bacillus Pyocyaneus</i>	33.33	50.00	58.33	25.00
<i>Bacillus Proteus Mirabilis</i>	41.67	66.67	66.67	41.67
<i>Escherichia Coli</i>	41.67	75.00	66.67	50.00
<i>Staphylococcus Aureus</i>	50.00	83.33	75.00	58.33
<i>Erysipelotrix Rhusiopathiae</i>	50.00	91.67	75.00	66.67

Table 7
RELATIVE INDEXES OF DEATHS IN THE
EXTENDED STUDY

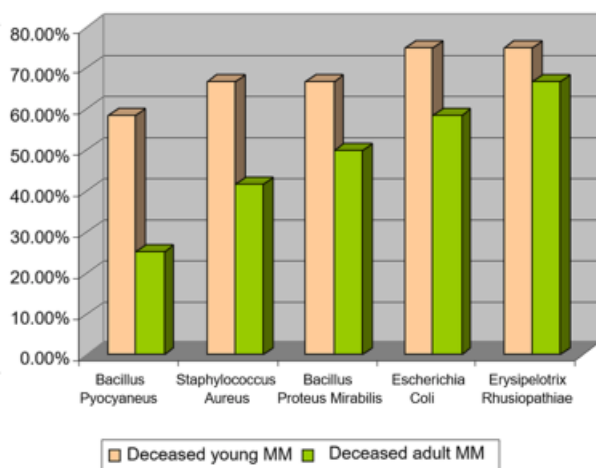


Fig 2. Relative indexes of death depending on the age of the inoculated MMs

physiopathological mechanism present in human pathology.

Comparative medicine is founded on the concept that other animal species share physiological, behavioural, or other characteristics with humans [32]. Compared with humans, MMs are remarkably less sensitive to the toxic or lethal effects of LPS (endotoxin, bacterial lipo-poly-saccharide) [17]. The LD₅₀ dose of LPS in MMs is about 1000-fold to 10 000-fold greater than the dose of LPS that is required to induce severe illness and hypotension in humans [17, 33, 34]. For the experimental induction of septic state, it is necessary to include more parameters like: the receptivity of the species for the microbial agent, the inoculation way, the germ dose, age. The sex of the experience animal is relevant only in the situation when the inoculated germ determines modifications at the level of the genital organs or can influence pregnancy [25, 35]. None of the inoculated germs in this study do not determine such modifications, this is the reason why the MMs were not distributed according to gender. Determination of LD₅₀

Table 8
CALCULATION OF LETHALITY INDEX OF MM

Etiological agent	Number of deaths MM				L %			
	without lesions	with lesions	young	adults	without lesions	with lesions	young	adults
1	2	3	4	5	6	7	8	9
<i>Bacillus Pyocyaneus</i>	4	6	7	3	15.4	17.6	17.5	10.0
<i>Bacillus Proteus Mirabilis</i>	5	8	7	5	19.2	23.5	17.5	16.7
<i>Escherichia Coli</i>	5	9	8	7	19.2	26.5	20.0	23.3
<i>Staphylococcus Aureus</i>	6	10	9	7	23.1	29.4	22.5	23.3
<i>Erysipelotrix Rhusiopathiae</i>	6	11	9	8	23.1	32.4	22.5	26.7
TOTAL	26	34	40	30	100	100	100	100

is a method with great accuracy and corresponds to the maximum dose that kills, in the given interval, 50% of the inoculated animals [25]. Quantitative bacteriological determination of the pathogeny of germs by LD₅₀ represents a fundamental method in the laboratory diagnosis, in the establishment of the role of the isolated germ in the case of an infection and at the same time offers the possibility to titrate some biological products used in curative and prophylactic purpose [17, 25, 34].

In our study, it was chosen the statistical method χ^2 (chi squared) for measuring the difference between the series of variation as a result of MMs inoculation with the etiological agent with the dilution corresponding to LD₅₀. This method is applied in those situations when the expected events exclude each other, meaning that it is possible to produce only one of them: to die or to survive. The problem is whether the differences of mortality are essential due to the age of MMs (young-adults) or/and to lesions done or these differences are random. The calculation of χ^2 is done based on the quadruple table. Only absolute digits are used. Our research took into analysis (for an easy calculation) homogeneous groups of MMs, and it was chosen the critical dilution in order to create the same conditions of study, meaning the same mortality at young MMs (of 50%). For one degree of freedom, we can say with X probability (calculated as a percentage) that there is a definite and not accidental (i.e. random, non-causal) difference between MMs age and the presence of traumatic lesions (with lesions or without lesions), this fact ultimately leading to a different mortality rate in the MMs groups. This way, the critical dilutions for every inoculated microbial agent were obtained.

Results referring to LD₅₀ show that the duration of the infectious process and the survival period of MMs depend on the infectious microbial agent (the inoculated strain), the inoculation pathway, the dilution of the culture suspension, associated lesions, age. By taking into consideration the critical dilution value as a set point it was noted that: the administration of a dilution containing suspensions of microbial cultures lower than the critical value determined the occurrence of a mild form of sepsis (meaning that the clinical manifestation of the infection was compatible with life) and the administration of a dilution containing suspensions of microbial cultures higher than the critical value causes death of the MMs by installing phenomena specifically involved in generating septic shock and MSOF. These modifications are in concordance with the elements identified at the histopathological examination done on the collected tissue fragments when the MMs were autopsied.

Conclusions

The gravity of the clinical symptomatology and the survival period of the MMs depend on the microbial species of the inoculated strain, the dilution of the suspension

culture, the presence of associated lesions, age. The administration of a dilution of suspension of microbial culture lower than LD₅₀ determines the occurrence of clinical manifestation compatible with life, and the administration of a dilution of suspension of microbial culture higher than the critical dilution produces death of MMs. In decreasing order, according to the value of LD₅₀, the most virulent strains are: *Erysipelothrix Rhusiopathiae*, *Escherichia Coli*, *Proteus Mirabilis*, followed by *Staphylococcus Aureus* and *Pseudomonas Aeruginosa*. The lethality index increases proportionally with the virulence of the ethologic agent and the presence of the associated lesions, confirming once again the similarity with physiopathological mechanisms that exist in human pathology.

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