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Investigation of Microbiological, Physiological and Histopathological Changes in Polymicrobial Infection in Old Rats

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ABSTRACT

Background: The aim of this study is to examine the microbiological, clinical, physiological and pathological findings of natural polymicrobial infection developing in a wistar rat colony under long-term observation.

Methods: In this study, 70 male Wistar albino rats, 3 years old, raised by conventional methods and housed in a cage environment were used. Thirteen rats with clinical symptoms such as torticollis and rotation were sacrificed for microbiological and histopathological examination after 14 days. For microbiological examination, the rapid diagnostic kit BBL Crystal system was used to identify bacterial agents in tissue and stool samples, and the traditional culture method was used for isolation.

Result: As a result of microbiological analysis, the rarely isolated *Staphylococcus capitis* (*S. capitis*) was isolated. Different *Staphylococcus* spp. It has been determined that the species can cause suppurative meningoencephalitis as well as septicemia in the brains of experimental animals., it is thought that polymicrobial infection will be useful for the identification of bacterial agents and infection prevention and treatment strategies.

Key words: Physiological and pathologic findings, Polymicrobial infection, Rat, *Staphylococcus capitis*.

INTRODUCTION

Laboratory animals are widely used for research in different medical disciplines such as modeling human anatomy and physiology, elucidating the physiological mechanisms of human diseases by experimental creation, investigating their therapeutic bases and neurobiological research (Kilkenny *et al*., 2009). Domestic rats live about 2 to 3.5 years (Pass and Freeth, 1993). Male Wistar rats can live up to 4.5 years (Lares-Asseff *et al*., 2006). Aging is a normal process in which there are biological, chronological and social changes that include all irreversible changes in physiological and mental states. Physiological and physical changes that occur with aging, limitation in daily living activities, depressive mood and behavioral disorders can be observed due to deterioration in cognitive functions (Altun *et al*., 2007). Elderly rats are used as a promising model for understanding the effect of memory and brain on age in humans (Gallagher and Pelleymounter, 1988). For this purpose, health checks of animals should be done regularly and followed because experiments should be carried out on healthy animals. There are many infectious agents seen in laboratory experimental animals. Bacterial infections are one of the most diverse and common infectious agents among these agents. Acute and chronic infections with bacterial agents affect experiment flow, quality and results. Identifying acute and chronic bacterial agents that can be seen in laboratory animals under observation is of strategic importance in order to achieve success in the prevention and treatment process. Bacterial agents that cause common microbial disease in rats, *Bordotella bronchiseptica, (B. bronchiseptica), Clostridium piliforme* (*C. piliforme*),*Corynebacterium kutscheri,* (*C. kutsheri*), *Helicobacter spp., Mycoplasma spp., Pasteurella spp., Salmonella spp., Streptobacillus miliniformis* (*S. miliniformis*), *Streptococcus pneumonia* Cilia- associated respiratory *Bacillus, Staphylococcus spp., Enterococcus*

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spp., Erysipelothrix rhusiopathiae (*E. rhusiopathie*), *Haemophilus spp., Klebsiella pneumonia* (*K. pneumonia*), *Leptospira icterohemorrhagiae, (L. icterohemorrhagiae*), (Kohn and Clifford, 2002). Some of these bacteria can show polymicrobial progress and be isolated. *S. aureus* is an agent with high incidence; morever other *S. haemolyticus, Staphylococcus., xylosus (S. xylosus), Staphylococcus. sciuri (S. sciuri) and Staphylococcus. cohnii (S. cohnii)* rat are the other staphylococcus species isoilated from mice. These species cause abcess, pneumonia and sometimes polyarthritis in rats and mice; dermatitis in gerbils. These agents cause lesions characterised with abcess in skin and lymph nodes, lesions in upper and lower respiratory tracts and sometimes generalised infections like septicaemia. In rats they can cause severe itching on shoulder and nape skin in a diameter of 1-2 cm area and ulcerative dermatitis characterised with watery-moisturised ecsamatous lesions (Ayyal, 2019). *S. capitis* is a factor that causes bacteremia along with endocarditis in mitral and tricuspid valves after prosthesis applications. It has been reported that approximately 20% of neonatal sepsis cases are associated with *S. capitis* (Stenmark, 2019). Coagulase-negative *Staphylococc*i are an important cause of delayed septicemia leading to mortality and morbidity, especially in infants with low birth weight. *S. capitis* has been reported to be sporadic (Butin *et al*., 2019). *S. capitis,* there is limited literature in the veterinary field. Unilateral massive scrotalpyocele associated with *S. capitis* has been reported in only one ram (Lacasta, 2009). Although the pulse rate and respiratory rate, which are among the physiological parameters of adult rats, vary at least 50% according to the movements of the animal and the individual, they have average physiological values of body temperature (BT): 38°C, heart rate (HR): 350 bpm (breaths per minute), respiratory rate (RR): 90 bpm (Tinsley *et al*., 2010). In addition to clinical parameters, physiological parameters are also used in the prediction and evaluation of bacterial infection (Hsu *et al*., 2021).

Sepsis is a life-threatening condition where the body's response to infection can reach failure of its own tissues and organs. One of the physiological parameters evaluated in the diagnostic criteria for defining sepsis is increased (>38°C) or decreased (<36°C) body temperature (BT), hyperventilation (respiratory rate (RT) >20 breaths per min or an arterial partial pressure of carbon dioxide <32 mmHg) (Levy, 2003). In rats, however, there are not any literatures on natural infection accompanied by sepsis. Apart from *S. aureus* other staphylococcal species cause subacut and chronic infections rather than sudden and severe nature. In addition to the factors from the bacterium (adhesion factors, toxin, graduating exoenzymes *etc*.), factors related with the host are also available in the development of these kinds of infections. *S. capitis*, has been found in hospitals in recent years and emphasis has been placed on the need to monitor it (Ding *et al*., 2020). Acut peritonitis case caused by *S. capitis* was reported from a periton-dialysis patient (Basic-Jukic, 2017). Rapid and accurate identification of *S. capitis* in the patients with endocarditis is of improtance for obtaining better prognosis and blocking the spread of this multidrug resistant bacterium (Firdaus *et al*., 2019). In this study, in rats frequently preferred laboratory experimental animals, microbiological, clinical, macroscopic and histopahologic findings in bacterail infections of polymicrobiological ethiology, *S. capitis* having the first priority developing naturally and spontaneously. After long term monitoring, central nervous system (CNS) involvement is recognised by torticollis and menage movement and in these rats to determine the variability in infections including *S. capitis* in microbiological examinations and findings in CNS and other organs is aimed. Although the molecular mechanisms of some polymicrobial infections are known, other polymicrobial diseases are not fully understood. Due to their complexity, the study of polymicrobial infections, a multidisciplinary approach and animal models studies are important. In this context, it was aimed to examine the microbiological and clinical, physiological and pathological findings of natural polymicrobial infection developing in a wistar rat colony under long-term observation.

MATERIALS AND METHODS

Before starting the current study, ethics committee approval was obtained from the Animal Experiments Local Ethics Committee (02/22/2018-2/24). The study was carried out in line with ethical principles and rules by protecting animal welfare and rights. The animal material of the study was 70 Wistar albino male rats, 3 years old, raised conventionally and housed in a cage environment and controlled conditions. During the trial period, rats were created in breeding cages of 45 cm ×28 cm ×17 cm in the experimental animal's production unit in the experimental animal's research center, with one animal in each cage. During the trial period, they were provided with water that they could drink at all times, refreshed daily and a 12-hour light/dark cycle was applied. The raw material and nutrient compositions of the rations of the rats in the experiment are presented in Table 1. BT of the rats were measured rectally with a digital thermometer (PlusMed, Biyomax-0614) between 9:00 and 10:00 in the morning. HR and RR were counted with the help of a stethoscope (Erkaphon 543 000, Erka, GERMANY). Among 70 rats kept under long-term observation, 13 rats with clinical symptoms of torticollis and rotation were sacrificed for microbiological and histopathological examination after 14 days. During systematic necropsy, all findings in the organs were evaluated macroscopically in terms of criteria such as color, shape, consistent and distribution of the lesions. Procedures and 5µm thick sections were prepared from paraffin blocks. In histopathological examinations meningitis, perivascular cellular infiltrations, neuronal degeneration and gliosis in cerebral and cerebellar sections and were graded semiquantitatively as; (-): no lesions, (+) mild, (++) moderate, (+++) severe.

Microbiological examinations

From heart, liver, spleen, lung, cerebrum and gaita samples Blood agar, Mac Conkey Agar, Nutrient Agar cultivations

were made and aerobic and microaerophylic atmosphere incubation was allowed. Bacterioscopy of the growing colonies was conducted identification of them was carried out. For identification, rapid diagnostic kit BBL Crystal system was used.

Statistical analysis

All the numerical data obtained as a result of the research were examined with the Pearson Correlation test in the IBM SPSS 23.0 statistical program. All significant differences were evaluated by testing at the $p<0.05$ level.

RESULTS AND DISCUSSION

Clinical and physiological findings

It was determined that the rats kept under observation exhibited self-rotation, coordination and symptoms of torticollis, contamination in the rectal regions due to fecal and urinary incontinence, restless, frightened, nervous and aggressive behaviors. In the following period, symptoms of inability to stand up, turning the neck 30-45°C to the left from the body axis, repetitive tremors and ventrodorsal movements, gait and posture abnormalities, involuntary motor movements, focal neurological findings and neuromotor abnormalities were detected. In Table 2, the Pearson correlation coefficient and P (sig.) value of the relationship between time (measured on days 0 and 14) and BT, HR and RR of 13 rats under observation are given (Karagöz, 2019). When the physiological findings are examined, it is seen that there is a positive relationship between the BT, HR and RR frequencies and as the day time increases, the body temperature, heart and respiratory frequencies of the animals also increase.

Clinical and physiological findings are parameters that can benefit the evaluation of the effects of polymicrobial infection and sepsis. Our present study is consistent with studies showing that BT, HR and RR increase due to infection (Martineau and Shek, 1996).

Pathological findings

Macroscopic findings

In macroscopic examinations of rats severe anemia, enlargement of liver and spleen, marginal blunt formation together with multifocal dull grey areas recognised from cortex were characterised. Lungs were generally dull and emphisematous. There were no clear macroscopic findings in cerebrum and cerebellum, while meningeal vessels were conjested and the hemisphere was mildly swollen.

Histopathojical findings

Central Nervous System; Non purulent meningitis having mostly macrophages was seen in vessels of meninges and brain paranchyma extensively with conjestion and haemorrhagies. In areas of the parietal cortex, cerebellum and hippocampus areas blood vessels were swollen and some inflammatory cells were seen in their lumen. Apart from three cases, mostly in the middle cerebrum and cerebellum basalis, the second areas of demyelination were characterised by sharp-edged, and empty vacuoles affecting the grey matter while in some areas of demyelination, microglial cell profileration and microglial cells were observed in the structure of spongiosis and in almost the entire grey matter. Again in the areas of pariatal hemisphere cortex and mid cerebrum intensive multifocal gliosis, neuronal degeneration and necrosis in grey matter. Findings in pons and medulla were not remarkable (Table 3).

In lungs interstitiel mononuclear cellular infiltration, peribronchial cuff and cubic formation in alveol epitels along with severe emphisema findings were observed suggesting

Table 1: Composition of experimental diet.

Ingredients	g/kg
Maize	315.0
Wheat bran	210.0
Soybean meal (% 46 protein)	170.0
Sunflower meal	112.0
Alfafa flour (% 17-19)	100.0
Soybean whole	50.0
Premix[1]	20.0
Limestone	15.0
Calcium propionate	5.0
NaCl	3.0
Total	1000
Analysed composition (% dry matter, DM) (per kg)	
ME, kcal/kg*	3213.2
Dry matter	886.9
Crude protein	200.0
Ether extsract	46.58
Crude ash	90.40
Crude fiber	97.89

[1]Premix provided per kilogram of diet: Vitamin A (retinol) 480.000 IU, Vitamin D3 (cholecalciferol) 100.000 IU, Vitamin E (α tocopheryl acetate) 800 mg, Vitamin K3 (menadione sodium bisulfite) 120 mg, Vitamin B1 (thiamine HCl) 40 mg, Vitamin B2 (riboflavin) 240 mg, Vitamin B6 (pyridoxine hydrochloride) 120 mg, Vitamin B12 (cyanocobalamin) 0.80 mg, Niacin (nicotinic acid) 1.200 mg, Vitamin B5 (Ca-D-pantotenate) 320 mg, Vitamin B9 (folic acid) 40 mg, Cholin 12.000 mg, vitamin H (D-biotin) 2 mg, Fe (iron carbonate) 1.000 mg, iodine (calcium iodate) 24,8 mg, copper (copper sulfate) 200 mg, copper (organic copper) 50 mg, manganese (manganese oxide) 4.000 mg, manganese (organic manganese) 200 mg, zinc (zinc oxide) 2.380 mg, zinc (organic zinc) 200 mg, selenium (sodium selenite) 8,10 mg.

Table 2: Physiological findings of the rats included in the study.

Parameters	Pearson correlation coefficient	P (sig.)
BT (°C)	0.954	0.000
HR (bpm)	0.973	0.000
RR (bpm) 0.975	0.000	

*BT: Body temperature; HT: Heat rate; beats per minute; RR: Respiratory rate; bpm: Breaths per minute.

inerstitiel pneumonia. In three cases edema and macrophages with hemociderin in alveol lumens. In spleen, rearkable hyperplasia in Malphigi corpucules and increase in sinusoidal macrophages were seen. In kidneys in addition to cortical focal haemorrhages, glomerular capillaries were swollen to fill Bowmann space and in cortex, multifocal non-purulent intersititiel macrophage and lymphocyte infiltration were observed.

Microbiological findings

After bacteriological examnination of organs, various bacterial identifications were made. Bacteria identifed and organs from which the bacteria were isolated are shown in Table 4.

In this study, 13 rats showing clinical symptoms such as torticollis and turning around themselves in laboratory experimental animal unit were examined microbiologically and pathologically. After microbiological examinations by cultures from brain, *S. xylosus* (6), *S. capitis* (5), *A. urinae* (3), *S. cohnii* (2); from heart, *Candida spp.* (3), *S. xylosus* (3), *S. capitis* (2); from liver, *A. urinae* (2); *S. cohnii* (2) and *Corynebacterium pseudogenitalum* (C. pseudogenitalum) (2); from kidneys *S. xylosus* (2); from gaita *Escherichia coli* (*E. coli*) (11), *S. simulans* (7), *S. sanguis* (4), S. *capitis* (2) and from blood *S. capitis* (1); from gaita *E. coli* (12), *S. simulans* (7), *S. sanguis* (4) and *E. durans* (4), *S. capitis* (2), *Pseudomonas spp.* (1) were isolated and indentified. In blood cultures, isolation of *S. capitis* from one animal showed the ocurrance of septicaemia (Table 5).

In rats bacterial occurrance and isolations together with neurological symptoms are mostly *S. capitis* and *S. xylosus*. These agents were found localised most frequently in brain and in other visceral organs. However the neurological symptomes observed were conidered associated with central nervous system localisation rather than the symptomatic expression of a general septicaemic findings. In fact, it was concluded that the findins observed as in the early stages of inflammation, were conjestion and meningitis next. However, in the cases at later stages conjestion, the sign of acut cases abated and infiltrations with mostly perivascular mononuclear cells ocurred instead. In some other cases conjestion was accompanied by degenrations. However, these degenerations localised in cerebrum and cerebellum were in neuron nuclei and cytoplasma and were mild. This picture of mild character was accompanied by necrosis in some cases or necrosis took place completely; neurons dissappearance was observed. While this kind of degenerative changes occurred in neurons (degeneration, necrosis), on the other side multifocal or diffuse areas of oligodendroglia and astroglia cells were observed. In this context, response by the organism with regenerative changes, the infection in fact as seen from symptomatic side, it was seen not to harm cerebrum and cerebellum that much and not fatal. On the other hand as persistent damages, occurrence of myelin loss (demyelination) under substantia alba was noted an important finding. In the margins of these areas microglia cells for phagositosis of myelin pieces an other glia cell types for the reparation of in the area were seen. Although it is not fatal, these central nervous system damages are caused by especially polymicrobial ethiology and when other organs are affected, more serious symptoms occure. It can be estimated that mortality risk can increase in this kind of situation. In central nervous system of experimental animals as in the bacterail infections, encephalomyelitis and demyelination producing some viral infections are known. Theiler's Murine Encephalomyelitis Virus is one of them. In immun competent mice it is a natural pathogen encephalomyelitis agent (Tsunoda *et al.,* 2016). Murin Hepatitis Virus (MHV), on the other hand is another natural payhogen in mice. Particularly the gliatropic MHV-A59 strain of MHV, causes chronic inflammation and demyelination in CNS (Matthews *et al*., 2002). Semliki Forest virus (SFV), can cause infection in neurons and oligodendrocytes. Demiyelinations develops

Table 3: Evaluation of histopathological findings in terms of congestion, gliosis, demyelination, meningitis, neuronal degeneration/ necrosis of the rats forming the study groups.

Case no/ Findings	Conjestion	Gliosis	Demyelination	Meningitis	Neuronal degenerastion/ nekrosis
1	+	+	_	+	-
2	++	++	++	++	++
3	++	+++	+++	++	++
4	+	++	++	++	+
5	++	+	++	++	+
6	+++	+++	++	+++	++
7	++	+++	+++	++	++
8	-	++	-	+	-
9	+++	++	+	+++	+++
10	++	+	-	-	++
11	+	++	++	+	+
12	+	++	+	+	+
13	++	+	+++	+++	++

Table 4: Evaluation of bacteria and microbiological findings in the organs from which bacteria were isolated in rats constituting the study groups.

Sample no	Brain	Heart	Liver	Spleen	Lungs	Kidneys	Gaita	Blood
1	*S. capitis*						*E. coli*	
2	*S. capitis*	*E. coli*					*E. coli*	
							E. durans	
							S. capitis	
3	*S. capitis* spp.						*E. coli*	S.capitis
	Pseudomonas						*E. durans*	
	S. capitis							
4	*S. capitis*						*E. durans*	
5	*S. capitis*	*S. capitis*					*E. coli*	
							Pseudomonasspp.	
6							*S. capitis*	
							E. coli	
							E. durans	
7	*S. xylosus*	*Pasteurellaspp.*	*S. xylosus*		*S. xylosus*		*E. coli*	
	A. urinae	*Candidaspp.*	*A. urinae*		*C. pseudogenitalum*		*S. sanguis*	
			C. pseudogenitalum		*L. pseudomesenterioides*		*S. simulans*	
8	*S. xylosus*	*Candidaspp.*	*A. urinae*			*Candidaspp.*	*E. coli*	
							S. simulans	
9	*S. xylosus*	*S. xylosus*		*S. xylosus*	*A. urinae*		*E. coli*	
		Candidaspp.			*Pasteurella spp.*		*S. sanguis*	
					C. pseudogenitalum		*S. simulans*	
					S. cohnii			
10	*S. xylosus*					*S. xylosus*	*E. coli*	
	A. urinae					*Pasteurellaspp.*	*S. sanguis*	
							S. simulans	
11	*S. xylosus*	*S. xylosus*					*E. coli*	
	S. cohnii L.	*Pasteurellaspp.*					*S. sanguis*	
	pseudomesen	*A. urinae*					*S. simulans*	
	terioides							
	A. viridans							
12	*S. xylosus*	*S. cohnii*		*L. pseudomes*	*S. cohnii*	*S. xylosus*	*E. coli*	
	A. urinae	*C. pseudogenitalum*		*enterioides*		*C. pseudogenitalum*	*S. simulans*	
	S. cohnii							
13		*S. xylosus*	*S. cohnii*	*S. cohnii*			*E. coli*	
			Pasteurellaspp.A.				*S. simulans*	
			urinae					

Table 5: Number of isolated and identified bacteria in the organs of the rats constituting the study groups.

Brain	Heart	Liver	Spleen	Lungs	Kidneys	Gaita	Blood
S. xylosus (6)	*Candida spp.* (3)	*A. urinae* (2)	*S. xylosus* (1)	*S. cohnii* (2)	*S. xylosus* (2)	*E.coli* (11)	*S. capitis* (1)
S. capitis (5)	*S. xylosus* (3)	*S. xylosus* (1)	*S. cohnii* (1)	*C. pseudogenitalum* (2)	*C. pseudogenitalum* (1)	*S. simulans* (7)	
A. urinae (3)	*S. capitis* (2)	*C. pseudogenitalum* (1)	*L. pseudomese nterioides* (1)	*S. xylosu s* (1)	*Pasteurellaspp.* (1)	*S. sanguis* (4)	
S. cohnii (2)	*Pasteurellaspp.* (2)	*Pasteurellaspp.* (1)		*L. pseudomese nterioides* (1)	*Candida spp.* (1)	*E. durans* (4)	
Pseudomona sspp. (1)	*C. pseudoge nitalum* (1)					*S. capitis* (2)	
L. pseudomes enterioides (1)	*E. coli* (1)					*Pseudomona sspp.* (1)	

spontaneously after 2. week of encephalytis, In this context, adult C57BL/6 and BALB/c mice are considere susceptible to the infection (Fazakerley and Walker, 2003). Sindbis virus (SV), is enaother infection seen in especially LSU mice. The infection may be assocaiated with denyelination (Kuramoto, 2019). In the light of this information, our study took only the bacteriologic-polimicrobial aspect of the issue and made evaluation, however, that the viral diseases develops spontaneously or had a subclinical character was not described further. This may be a limitation for the study. In our study findings were observed in almost all animals from mild to severe gliosis and in 10 animals mild or severe demyelination. Therefore there might be ethiological agents running together with polymicrobial ethiology in our study with the possibility that viral infections may cause this situation at the same time. Thus, it is considered useful to review the histopathological findings in other organs as well other than the brain more clearly and to compare the findings in mixed character polyfactorial ethiology. This will provide us to review the spontaneous and subclinical infections in experimental animals in several aspects.

CONCLUSION

In conclusion, similar findings between *S.capitis* and *S.xylosus* were mostly isolated with brain lesions and neural symptoms suggest the subject of our study. However, in some cases, different bacteria have been isolated from the brain. These agents have also been isolated from other organs. Evaluation of such subclinical and over time infections in rat colonies may not be possible in a short time. During the study, autopsy was performed even on rats with neurological symptoms and dullness symptoms at first, but the stage of infection could not be determined. Although there are parameters that will be beneficial in the evaluation of physiological parameters (CT, HR and RR) during the course of the infection, microbiological isolations should be emphasized. Among them, *S. capitis* and *S. xylosus* are particularly sought after. On the other hand, long-term observations are needed when infection is investigated on a colony basis rather than individually. In this context, more comprehensive studies on rats with neurological symptoms are needed. It is thought that our current results will contribute to researches in the field of prevention and treatment by determining the pathogens involved in the etiology of polymicrobial infections.

Conflict of Interest

The authors declare that there is no conflict of interest.

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