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Florula of Larval and Imaginal Phases of the Volfartova Fly (*Wohlfartia magnifica*) In the Conditions of the Steppe Zone of The Pavlodar Region.

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ABSTRACT

Groups of bacteria were found during research in a steppe zone of the Pavlodar region, belonging to 3 families: *Bacillaceae*, *Micrococcaceae*, *Enterobacteriaceae*. 13 species of pathogenic and opportunistic bacteria are obtained and identified, which cause diseases. Reception of agents from flies of *Wohlfartia magnifica* family in region farms forces to pay attention to quite real possibility and contagion of various infections. It creates the menacing epidemiological and epizootiology situation on the adjacent to farms of populated places, as flies with excrements can infect forages and migrate on considerable distances.

Keywords: bacteria, diseases, infections, larvae, microorganisms, flies, sheep, pathogenic microorganisms, carriers.

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INTRODUCTION

Flies are known as carriers of causative agents of dangerous infectious and invasive diseases. Therefore, in the populated places and on the pastures, studying of microbial and helminthosis impurity of flies represents scientific and practical interest.

Epidemiological value of flies was opened by E.N. Pavlovskiy and V.P. Derbeneva-Ukhova, they participate in distribution about 70 pathogenic microflora, and including agents of a tularemia, anthrax, diphtheria, cholera, plague, a crab hand, etc. [2; 8; 12].

At researches of the room flies, which were caught in various places on separate individuals, by E.N. Pavlovskiy, it was different various quantity of bacteria: from 550 to 6,6 million, and in intestines of flies – from 16 thousand to 28 million bacteria. Absolute cultures of such bacteria, as, for example, a livid-purulent bacillus, colibacillus, typhoid fever, etc. were got from flies [12].

Ability of flies to extend various infectious diseases depends on the following reasons: structure of proboscis; ability of various pathogenic bacteria to match to the digest effect of digestive juice of intestines of a fly; they sit down directly on food at often flights from the infected subjects, etc.

The room fly plays the greatest role in distribution of causative agents of highly infectious intestinal diseases and first of all a typhoid, tuberculosis. They devour tubercular sticks of patients, and Kokhov's bacilli remain live most long than other, which aren't forming spore of microorganisms as in the fly (16 days) and in her excrements (15 days). The most important way of transfer of this infection is impurity by the fly Kokhov's sticks of food and drink as at contact by tarsuses, and taking-up of all crawl or ejection excrements. Tubercular sticks about two months live in the dried "flies" spots. Being pounded in dust, these spots gave to air the sticks consisting in it, which can be inhaled by healthy people [1].

Flies are involved in spread of diphtheria from other infectious diseases. The fly can transfer also causative agents of wound abscess, anthrax, various eye diseases, in particular of a suppurative infection of eyelids and trachoma. [13].

Fly harms is aggravated by dispersion of eggs of various a worm, which were ate with excrements by the patients, such as: ascarids, pinworms, *Trichuris trichiura*, tapeworm, tapeworm unaided, tapeworm dwarfish, hookworm of a duodenum and pathogenic protozoa: cyst of dysenteric amoebas, lamblia, etc. [7; 12].

Pollution bacterial rate and seasonal change of these indicators at flies depending on density of population of the above mentioned insects, and also sanitary and economic value of this phenomenon were established by Zh.M. Isimbekov and N.B. Sarsembayeva at Priirtyshiyev of Semipalatinsk region on milking platforms and in summer pastures for cattle. Researches among flies on bacterial impurity by L.T. Bulekbayeva, Zh.M. Isimbekov and etc. were carried out in the conditions of poultry farms, in Semipalatinsk city [4; 9].

The bacterial composition of flies was investigated by M.S. Ishekenov in the Northern Kazakhstan. However such researches for the last 20 years in a due measure weren't carried out. Considering it, we considered necessary to make research of bacterial impurity of flies in farms of Pavlodar region. [10].

MATERIAL AND RESEARCH TECHNIQUE

Material for carrying out microbiological and mycologic researches was collected in farms of the Pavlodar area and Uspenskiy area of the Pavlodar region. The experimental part of work was carried out in Pavlodar branch of the Republican State Enterprise "Republican Veterinary Laboratory" of Committee of Veterinary Control and Oversight of the Ministry of Agriculture of the Republic of Kazakhstan. During the period from May to October, 2012 for carrying out researches of flies it was collected in sterile test tubes in places of their greatest congestion from a surface of wounds of sheep, and the Volfartova's flies took larvae from wounds. For reliability of the received results the total mass of the studied object was taken per 1g that in quantitative recalculation was made 20 copies of imaginal individuals of Volfartova fly. [3].

The microbial seeding of the surface of a *Volfartova* flies and their larvae was revealed by the standard techniques [5; 6; 12; 15].

For definition of qualitative composition of microorganisms, the colonies were grouped in cultural, morphological and tincture properties. The specific composition of bacteria was established by means of the determinant of Bergi [14; 11]. The light microscope of a series of Nick Med-1 of "Lomo" XE 1360 was used, it was carried out photography of growth of microbes on various nutrient mediums of the colored microslide of bacteria, fungus.

For the purpose of establishment of bacterial impurity of flies we used 720 individuals of the imaginal phases of *Wohlfarthia magnifica* and as much larvae of this individual, collected in Uspenskiy and Pavlodar areas from sheep.

RESULTS OF RESEARCH

The groups of bacteria relating to 3 families are found during research: *Bacillaceae*, *Micrococcaceae*, *Enterobacteriaceae*.

As it was shown in table 1, the received microorganisms from outer covering and intestines of an imago and larvae of flies are different in morphological, biochemical properties and they are belong to various taxons. Among them bacilli were prevailed quantitatively, and also klostridiya, sporous and staphylococcus. The found microorganisms in farms of the Pavlodar area indicate not really safe sanitary and epidemiologic state and danger of the spread of the agent among animals and the population.

Apparently from the table of 1 bacterium from flies and larvae from Pavlodar and Uspenskiy area of the Pavlodar region, received by us, belong to 2 sections – *Firmicutes* and *Gracilicutes*, 2 classes – *Firmibacteria* and *Scotobacteria*, 3 families:

- *Bacillaceae* family is presented by 5 genres – *Bacillus*, *Clostridium*, *Desulfotomaculum*, *Sporolactobacillus*, *Sporosarcina*;
- *Micrococcaceae* family - with one genus *Staphylococcus*;
- *Enterobacteriaceae* family - with one genus *Proteus*.

The found bacteria belong to 13 types from which 12 specieses were gram (+), and 1 species of *Proteus vulgaris* is gram (-). Inoculation was carried out on millieus: beef-extract agar, Endo's agar, bismuth-sulfite agar, Ploskirev's agar, staphylococcal agar. The growth isn't revealed at inoculation on Endo's millieu, Ploskirev's, Bismuth-sulfite agar. On a staphylococcal agar there were colonies of dairy color. Clear growth of colonies of all 13 specieses were observed on BEA (beef-extract agar). Cultural and biochemical properties and growth form is described in table 1.

Found microorganisms were painted on Gram and Romanovskiy-Gimza which are shown in figure 1.

Apparently from the table 1 species of bacteria, received from external covers of larvae of flies in two areas, aren't differ by the first 7 specieses, the 8th species of bacteria in Pavlodar area is presented by a species of *Sporosarcina ureae*, and in Uspenskiy area the species of *Desulfotomaculum nigrificans*.

Specieses of bacteria from larvae viscus in these areas also have differences, so in Pavlodar area they are presented by specieses with 2 - 11 (table 1), and in Uspenskiy area from 2 on 7, and also 12.

From external covers of an imago of *Wohlfartia magnifica* in Pavlodar area we received bacteria from 2-8 (table 1), and in Uspenskiy area from 1-7 and 12, 13, and from internal according to 1-11 and from 1-7, 12, 13.

Table 1: Data of bacteriological researches of the cultures, received from *Wohlfartiyia magnifica* in Pavlodar area and Uspenskiy area

GENUS	Quantity of the used insects		Results of researches		
	larvae to	imago	Received cultures. Morphology	Color on Gram	Cultural properties. Growth on millieus (beef-extract agar (BEA), BEB (beef-extract broth), Endo, etc.)
Pavlodar area					
<i>Wohlfartia magnifica</i>	180	180	1. <i>Proteus vulgaris</i> Atropous sticks, they have 0,4-0,8*1-3 micrometers. They are Gram-negative, mobile.	Gr(-)	BEA – it is creeping growth with a thin transparent film
			2. <i>Bacillus subtilis</i> Atropous sticks to 10 micrometers have the rounded-off or stub ends, often in pairs or chains.	Gr(+)	BEA - Colonies are white or grayish, creeping on a wall test tubes, in condensate water a film
			3. <i>Bacillus pumilus</i> Atropous sticks to 10 micrometers have the rounded-off or stub ends, often in pairs or chains.	Gr(+)	BEA - The thick dead-white wrinkled film, which is formed a film in condensate water
			4. <i>Bacillus cereus</i> Gram-positive sticks, which sizes reach 0,5-2,5*1,2-10 micrometers. They are mobile. There are aerobes or facultative anaerobe bacterias. They are formed the central - located spores.	Gr(+)	BEA - Colonies are white, firm, round, sometimes painted in yellowish color, at the borders have gyrose threads
			5. <i>Bacillus megaterium</i> Atropous sticks to 10 micrometers, with the rounded-off or stub ends, often in pairs or chains. They are formed endospores.	Gr(+)	BEA - Colonies are mucous, yellow-white, are darkened in the presence of a tyrosine in the millieu
			6. <i>Clostridium sporogenes</i> Mobile sticks 3-6*0,5 micrometers in size with rounded off, sometimes with the acerous ends, often placed in couples or short chains.	Gr(+)	BEA – they are formed colonies of the wrong form with rizoid borders, they have gray color, convex
			7. <i>Clostridium perfringens</i> Large, strictly grams - positive, there are no ciliums, they aren't mobile (one of the few motionless species). Classical forms are presented by short sticks which have the ends with the stubbed at right angle from (0,6-1,0 × 1-1,5 micrometers)	Gr(+)	BEA – they are formed S- and R-colonies. S-colonies are round, sapful, dome-shaped, with glabrate and smooth borders, at the beginning of growth transparent, become later muddy, grayish-white.
			8. <i>Sporosarcina ureae</i> Cages are spherical or oval, 1-2*2-3 micrometers, they are generally in the form of diplococci and tetrads, but sometimes in the form of cubic packages	Gr(+)	BEA – they are well grown, forming colonies from cream to the orange colors
			9. <i>Staphylococcus aureus</i> Gram-positive coccus, to which in pure culture are peculiar the congestion in the form of grapes clusters.	Gr(+)	BEA – they are formed the muddy round equal colonies of cream, yellow or orange color.
			10. <i>Staphylococcus auricularis</i> Cages spherical, with a diameter of 0,5-1,5 micrometers, are single, in couples and in groups of the wrong form.	Gr(+)	BEA - colonies are usually opaque, white or cream, sometimes from yellow to the orange colors
			11. <i>Staphylococcus epidermidis</i> The motionless coccus with a diameter of 0,5-1,5 micrometers, which are placed in smears single, couples or clusters.	Gr(+)	BEA – there are whitish smooth convex colonies
Uspenskiy area					
<i>Wohlfartia magnifica</i>	180	180	8. <i>Desulfotomaculum nigrificans</i> Atropous or curved sticks, 0,3-1,5*3-9 micrometers, are usually single placed. Spores are ovoid, subterminal	Gr(+)	BEA – Colonies are white or grayish
			9. <i>Sporolactobacillus inulinus</i> Atropous sticks of 0,7-0,8*3-5 micrometers, single, in couples or they are rare in short chains.	Gr(+)	BEA – Colonies white or grayish, gyrose at the borders

Note: The first 7 species of bacteria meet and in Uspenskiy area (*Proteus vulgaris*, *Bacillus subtilis*, *Bacillus pumilus*, *Bacillus cereus*, *Bacillus megaterium*, *Clostridium sporogenes*, *Clostridium perfringens*)

The received cultures in Pavlodar area:

from larvae (external lavage) – 1-8;

from larvae (internals) – 2-11;

from flies (external lavage) – 2-8;

from flies (internals) – 1-11.

The received cultures in Uspenskiy area:

from larvae (external lavage) – 1-7, 12;

from larvae (internals) – 2-7, 12;

from flies (external lavage) – 1-7, 12-13;

from flies (internals) – 1-7, 12-13.

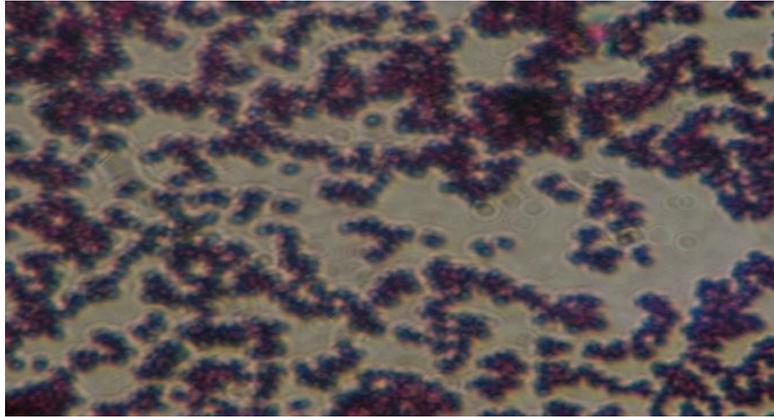


Figure 1: The found bacteria of genus *Staphylococcus*

The interrelation of microflora of an organism of flies with nature of food and feature of a substrate is shown by table 2. Volfartova fly generally eats nectar of plants in an adult form, but sometimes it is observed also alimentation by cadaveric remains. And apparently from table 2 from an imago flies, caught in Pavlodar area from wounds of sheep, we received the bacteria relating to 4 genuses – *Proteus*, *Bacillus*, *Clostridium* and *Staphylococcus*, and from the pascual platforms of sheep except this genuses are received *Sporosarcina* bacteria.

Species composition of a bacteria from the same objects in Uspenskiy area are a little differ, so at inoculation from wounds of sheep we received 4 genuses – *Proteus*, to *Bacillus*, *Clostridium*, *Desulfotomaculum*, and from the pascual platforms the first three specieses were identical, and the fourth species is presented by the genus *Sporolactobacillus*. Besides we carried out catching of flies from corpses of sheep and received 3 specieses of the bacteria relating to 3 genuses - *Bacillus*, *Clostridium* and *Sporolactobacillus*.

From the conducted researches we can do the following conclusions: the microorganisms causing diseases remain in an organism of arthropods for a long time, and they are discharged with their excrements, transferred trans-phased, where it is provided the circulation of activators in the nature. Studying of activators from flies of *Wohlfartia magnifica* family in farms of area is paid attention to quite real possibility and spread of various infections. It creates the menacing epidemiological and epizootology situation on the adjacent to farms peopled places as flies with excrements can pollute forages and migrate on considerable distances.

Thus we received the following microorganisms, pathogenic and opportunistic for the human and animals:

The species of *Proteus vulgaris* is opportunistic, is widespread in the soil, sewage, manure and causes in the person and animals pyoinflammatory diseases of an urogenital tract, sepsis, purulent damages of wounds and burns, osteomyelitis, meningitis and food toxic-infektion.

Bacillus subtilis is widespread saprophyte. It can cause diseases of the person as a part of microbial associates, especially at persons with immune frustration. Considerable part of the heroin sold by street

dealers is infected of *Bacillus subtilis*, from here they have heavy eye infections and bacteriemia at addicts and also such diseases as meningitis, pneumonia, eye infections and endocarditis are shown.

Bacillus cereus is widespread in the nature, causes sporadic cases of food poisonings at the person.

Bacillus megaterium is widespread saprophyte, causes bacteriemia, a septicemia, meningitis and food toxicin-infection.

Table 2: Microflora of external covers of Volfartova fly of the Pavlodar and Uspensky areas

№	Species of fly	Development phases	Quantity of the studied exemplars	Substratum and source of alimentation	Composition of the received microorganisms						
					<i>Proteus vulgaris</i>	<i>Bacillus</i>	<i>Clostridium</i>	<i>Sporosarcina</i>	<i>Staphylococcus</i>	<i>Desulfotomaculum</i>	<i>Sporolactobacillus</i>
Pavlodar area											
1	<i>Wohlfartia magnifica</i>	imago	180	From wounds of sheep	+	+	+	-	+		
2				from the pascual platforms of sheep	+	+	+	+	+		
Uspenskiy area											
3	<i>Wohlfartia magnifica</i>	imago	180	From wounds of sheep	+	+	+	-	-	+	-
4				From corpses of sheep	-	+	+	-	-	-	+
5				From the pascual platforms of sheep	+	+	+	-	-	-	+

Bacillus pumilus except pathogenicity for animals is a dangerous species at spread of diseases of bacteriemia, septicemia, meningitis and many others.

Clostridium perfringens is a spore generating bacterium, it is met in the soil, sewage, is the activator of food toxicin-infection at the person and animals, also the causative agent of malignant edema and gas gangrene, an infectious enterocsimiya of animals and anaerobic dysentery at young growth of farm animals.

Clostridium sporogenes is discharged not only from the soil, but also from excrements of sheep, dogs and at pathological conditions of the person and animals. Is the causative agent of malignant edema and can meet in wounds at wound infections.

Sporosarcina ureae are widespread in soils.

Staphylococcus aureus (golden staphylococcus) is widespread everywhere, is the causative agent of sepsis with metastatic abscess, causes conjunctivitis, it is found in skin pyoinflammatory processes. Staphylococcus can meet in medical institutions: gynecologic, surgical, somatic, stomatological hospitals, causing staphylococcal infections.

Staphylococcus auricularis is widespread everywhere, cause otitis or inflammations of the auditory canal.

Staphylococcus epidermidis is widespread everywhere, stimulates development of inflammatory reactions and has multilateral effect on tissues.

Desulfotomaculum nigrificans are widespread in the soil and other habitats.

Sporolactobacillus inulinus are discharged from forage of chickens and from the soil.

Also it is carried out the researches on existence of mushrooms of collected imago and larvae of flies, where we were discharged 3 species of mushrooms apparently from table 3. The fungus of species *Rhizopus sp.* is discharged in all tests of flies and larvae, it is found practically in all studied tests as it is a soil saprophyte and meets everywhere. It has feeble toxic properties against animals and insects. It is discharged from the studied tests 2 species of funguses of the genus *Aspergillus*: *Aspergillus fumigatus* and *Aspergillus flavus* which have toxic properties not only for insects, but also for warm-blooded, causing aspergillomycosis, which is shown in figure 2.



Figure 2: The fungus of species *Aspergillus*

Table 3: Results of mycologic researches of Volfartova fly of Pavlodar and Uspenskiy areas of the Pavlodar region

Species of funguses	Development phases	Place of gathering of insects		
		From wounds of sheep	From corpses of sheep	From the pascual platforms of sheep
<i>Rhizopus spp</i>	Imago	-	+	+
	larvae	-	+	+
<i>Aspergillus fumigatus</i>	Imago	-	-	-
	larvae	+	+	-
<i>Aspergillus flavus</i>	Imago	-	-	-
	larvae	+	+	+

Thus, the conducted research allows making the following conclusion.

CONCLUSION

Flies are widely spread and migrate to settlements, often are consumed various sewage, contaminating a body surface of microflora and plentifully swallowing them at meal. Therefore during all summer period the flies constantly are carriers of various microflora among which there are pathogenic groups.

From the Volfartova fly, caught in Pavlodar area, are discharged 11 groups of microorganisms, with prevalence of bacilli and staphylococcus, and there are 9 groups of microorganisms with prevalence of bacilli in Uspenskiy area. All microorganisms which contain in the decaying organic substratum, can appear on a surface and an organism of flies. The microflora of the milieu is richer, the more variously transferred by insects bacterial composition.

The greatest bacterial seeding of flies is noted in summer months, that is quite natural, as density of population of flies generally increases in the middle of summer.

A certain alarm is caused by toxic the funguses of species *Aspergillus*, which cause mass aspergillar diseases among animals through the infected forages, or at violation of preparations and storages of forages; as the got spores of the activator, having propagated, are caused at strong contamination of forages the mass mortality.

In general, these researches have practical value as they are observed the picture of danger to the population and production, as flies are contaminate of pathogenic microflora.

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