

Pathogenic Viruses, Fungi and Bacteria Detected in Wild Animals of Different Habitats

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ABSTRACT

A wide range of pathogenic viruses, fungi and bacteria have been identified in biomaterials from wild animals of 24 species living in the wildlife in Russia, including nature conservations, hunting farms, zoos and wild animal habitat centers. Epizootically significant bovine viruses e.g. rotavirus, coronavirus, infectious rhinotracheitis, parainfluenza-3, viral diarrhea viruses were detected in deer, mouflons, alpacas, monkeys, elephants, tiger. The clinically significant fungi of the genera *Candida*, *Geotrichum*, *Trichosporon*, *Aspergillus*, *Fusarium*, *Beauveria*, *Phoma* were isolated from skin lesions, respiratory tract, internal organs, muscle tissue, brain from wild animals of 14 species. Cultures of *Staphylococcus aureus*, *S. cohnii*, *Streptococcus parauberis*, *S. equi*, *S. suis*, and *S. bovis* were isolated from deers of different species and mouflons with respiratory disease. In some cases, there was a strong correlation between the detected pathogen and pathology, in others, this relationship was not obvious.

Keywords: Wild animals, Viruses, Fungi, Bacteria, Wildlife infectious diseases.

INTRODUCTION

Wildlife pathogen surveillance plays a significant role in risk assessments for humans, domestic animals, and wildlife. Diseases like rabies, anthrax, foot-and-mouth disease, and listeriosis in wild animals are notifiable diseases (Gortázar et al. 2022; Kuiken and Cromie 2022; Sarker 2022). However, the range of pathogens considered less dangerous or opportunistic, their potential to establish natural reservoirs, to cross species barriers, and their circulation among wild and domesticated animals remains poorly understood (Fabisiak et al. 2018; Blahove and Carter 2021; Pchelnikov et al. 2023; UI-Rahman et al. 2024).

In recent decades, a significant increase in the incidence of opportunistic mycoses has been observed among wild animals. Some of these diseases are highly contagious, can cause mortality and affect large populations of animals in the wild. Typical examples include chytridiomycosis in amphibians, white nose syndrome (WNS) in bats, and snake fungal disease (SFD) (Seyedmousavi et al. 2018). At the same time, the species

composition of the microbiota in wild animals both in Russia and abroad has been extremely insufficiently studied. In 2018, one of the first ecological and epidemiological studies to detect fungal pathogens in wild animals was undertaken in Brazil. Fungi were found in 102 of 1063 samples, including pathogenic species in 89 samples. This study emphasizes the importance of studying the mycobiota of wild animals, which is still only episodic (Losnak et al. 2018).

Factors impeding these investigations include challenges in collecting and transporting biological samples to laboratories, as well as a limited array of validated diagnostic tools. There is currently a pressing need to address this knowledge gap. Given Russia's vast territory, diverse wildlife, government initiatives for nature reserve development, and the growing popularity and commercialization of hunting, it is crucial to objectively evaluate the biosafety aspects of these activities. Our study involved virological, mycological, and bacteriological analyses of clinically relevant pathogens in Russian wildlife populations. The findings are presented herein.

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MATERIALS AND METHODS

Ethical approval missing

The object of study was the biomaterial from wild animals of 24 species living in natural conditions, nature conservations, in zoos, hunting farms and wild animal habitant centers of various regions in Russian Federation. Samples were taken from clinically healthy, suspected for infectious diseases, dead for an unknown reason and obtained by hunting animals.

The virological study was aimed at identifying viruses of parainfluenza-3 - *Respirovirus bovis*, infectious rhinotracheitis - *Bovine alphaherpesvirus 1*, bovine viral diarrhea - *Pestivirus bovis*, lumpy skin disease - *Capripoxvirus*, sheep pox *Capripoxvirus*, bovine *Rotavirus A*, bovine *Betacoronavirus 1*. Additionally, tiger biomaterials were examined for *Alphacoronavirus 1*, *Protoparvovirus*, *Felid alphaherpesvirus 1*, leukemia - *Gammaretrovirus*, feline immunodeficiency - *Lentivirus*. The main method of investigation was real-time polymerase chain reaction (RT PCR). DNA/RNA was extracted using kits produced by VetFactor. Amplification was carried out on Light Cycler 96 thermal cycler (Roch, Switzerland) using kits for genome detecting (Vet Factor, AmpliSens, RF) of corresponding viruses. Some samples were passed 3-5 times in the primary culture of calf kidney cells for virus isolation.

The mycological study included selected material cultivation on a differential diagnostic medium for dermatophytes DTM-Expert (VIEV, Russia) and Sabouraud medium with chloramphenicol (HiMedia, India). Incubation was carried out under aerobic conditions at 26-28°C. Cultivation continued until the formation of colonies with pronounced cultural and morphological features. The identification was carried out on the basis of the macro- and micromorphological properties of the isolates in comparison with the reference data of the mycological atlas "Atlas of Clinical Fungi" (de Hoog et al. 2020). In doubtful cases, clarifying identification was performed by MALDI-TOF mass spectrometry and ITS sequencing.

The bacteriological study included microscopy of Gram-stained smears, cultivation on blood agar with 10% of blood, Endo medium, cetrimid agar with glycerin, selective medium for isolation and testing of staphylococci, selective medium for isolation of streptococci, bile-esculin agar with sodium azide, McConkey agar. Incubation was carried out under aerobic and anaerobic conditions at 37°C. The biochemical characteristics of the isolated cultures were determined in the Staphy 24 and Strepto 16 PLIVA-Lachema tests. Hemolytic, coagulase, catalase activities, sensitivity to antibiotics were tested by conventional methods. The PathoDextra TM kit was used to identify the serogroup affiliation of streptococci.

RESULTS and DISCUSSION

Our research has revealed a wide range of clinically significant pathogens of various origin in wild animals in Russian Federation. Bovine rotavirus, coronavirus, parainfluenza-3, infectious rhinotracheitis and bovine viral diarrhea-mucosal disease viruses were detected in wild animals of 10 species as well researchers in other countries

(Jiménez et al. 2022; Kálmán et al. 2005; Pchel'nikov et al. 2023). These agents cause epidemics in cattle. Our research has shown that they are also capable of infecting a wide range of wild animals, including those not related to artiodactyls. The spectrum of viruses found in different species is shown in Fig. 1.

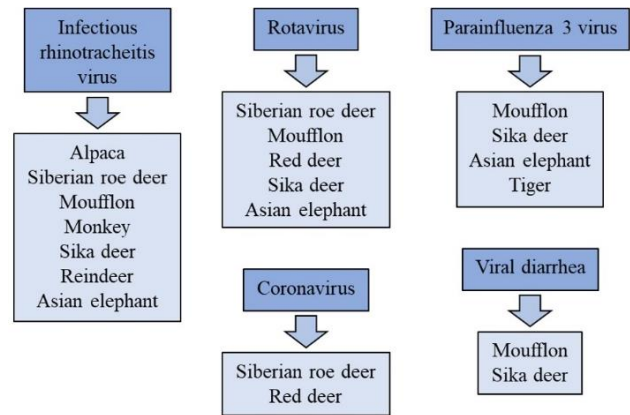


Fig. 1: Spectrum of viruses detected in wild animals.

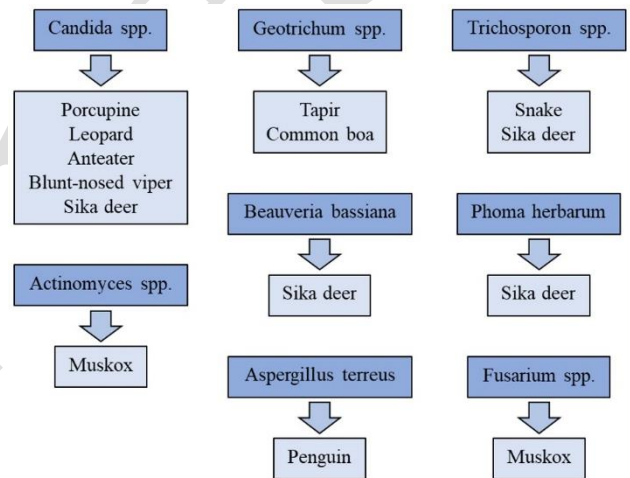


Fig. 2: Fungi isolated from wild and exotic animals.

Rotavirus was detected in Siberian roe deer (*Capreolus pygargus*), red deer (*Cervus elaphus*), moufflons (*Ovis aries*) with signs of damage to the respiratory and digestive systems in hunting farms. In single cases, rotavirus was found in combination with coronavirus, infectious rhinotracheitis and bovine viral diarrhea-mucosal disease viruses. Rotavirus in combination with parainfluenza-3 virus was found in a nasopharyngeal smear of one clinically healthy circus elephant (*Elephas maximus*).

The infectious rhinotracheitis virus was detected in one red deer, one sika deer (*Cervus nippon*), one moufflon with respiratory pathology in the hunting farm. This agent was also found in nasopharyngeal smears of a circus monkey with signs of mild rhinitis and clinically healthy circus elephant, 3 reindeer (*Rangifer tarandus*), 5 alpacas (*Vicugna pacos*) from farms.

The parainfluenza-3 virus was detected in the tiger (*Pantera tigris altaica*), which lived in natural conditions, as well as one sika deer, one moufflon in a hunting farm and a clinically healthy circus elephant. BVD virus was

detected in one moufflon and one sika deer, coronavirus – in one red deer and one siberian roe deer in a hunting farm. Moose that lived in natural conditions were free of all viruses included in the study. Biomaterials from all 10 animal species were free from pathogens of lumpy skin disease, sheep pox, orthopneumovirus. The viral spectrum in moufflons, roe deer, and sika deer was most diverse.

Rotavirus, coronavirus, parainfluenza-3, infectious rhinotracheitis and bovine viral diarrhoea-mucosal disease viruses were detected in postmortem samples of wild artiodactyls internal organs and tissues (Table 1). As can be seen from the Table 1, epizootically significant bovine viruses have the ability not only to infect, but also to cause the dissemination process in wild artiodactyls. The localization of the viruses corresponded to their tropism, except for the presence of rotavirus in the lungs and pulmonary lymph nodes.

The parainfluenza-3 virus was isolated from the submandibular and mesenteric lymph nodes of the tiger (*Pantera tigris altaica*), which died for an unknown reason. Due to the rare possibility of studying postmortem samples of this species, a PCR analysis for feline viruses was additionally performed. Coronavirus was found in the lungs, parvovirus – in the liver, submandibular and mesenteric lymph nodes, feline herpesvirus (FHV) – in the mesenteric lymph node. PCR for the genome of immunodeficiency and leukemia pathogens was negative.

Most of the positive findings are associated with lymphoid tissues. This reflects an active immune response to viral infection. A cultural mycological study showed a high prevalence of opportunistic pathogenic fungi in wild and exotic animals (Fig. 2). Sixteen fungal cultures were isolated from 14 different animals (78%), including 10 yeasts, 5 moulds and 1 actinomycete. Yeast fungi were represented by species of *Candida*, *Geotrichum* and *Trichosporon* genera, mycelial fungi by *Aspergillus*, *Fusarium*, *Beauveria*, *Phoma*. All detected fungi were considered clinically significant. In most cases, fungi were isolated from skin lesions (61%). *Aspergillus terreus* was isolated also from the penguin's airway sacs.

The isolation of fungi from the two dead sika deer (*Cervus nippon*) in a hunting farm deserves close attention. Impaired movement coordination, exhaustion, weakness, and reduced mobility were observed in the animals. Foci of thin strands with irregular shape and dark pigmentation were found on the costal pleura, brain, muscle tissue.

Phoma herbarum culture was isolated from pigmented areas of the brain, and *Beauveria bassiana* from unchanged areas. *B. bassiana* growth on the DTM-Expert medium was accompanied by red staining of the substrate and indicates the resistance of the fungus to cycloheximide. *Trichosporon lactis* was isolated from pigmented areas of muscle tissue. We were unable to find information about *Trichosporon* mycoses of muscle localization in the available literature. These fungi, as a rule, cause superficial opportunistic infections, but are capable of causing invasive and disseminated forms in cases of suppressed immunity (Almeida Júnior et al. 2016).

It can be assumed that the dark pigmentation of the brain areas was caused by pheohyphomycete *Phoma herbarum*, which has a dark brown pigmentation of the mycelium. Fungi of the *Phoma* genus are currently considered as rare opportunistic pathogens that cause mainly mycoses of surface tissues (Bennett et al. 2018). There are single cases of brain lesions in humans (Rai et al. 2022), but such cases have not been described in animals.

The isolation of *B. bassiana* from the deer brain is also of considerable interest. This species is known primarily as entomopathogenic, but single cases of deep mycotic lesions in humans have been described (Henke et al. 2002). Infections of *Beauveria spp.* in warm-blooded animals are not described in the literature.

At the autopsy of a fallen pregnant female sika deer, multiple rounded foci of white necrosis were found on the placenta. The yeast fungus *Candida parapsilosis* was isolated from the necrotic tissue of the placenta. The identification of these isolates was confirmed by ITS (internal transcribed spacer) section sequencing. The yeast fungus *Candida parapsilosis* isolated from necrotic foci of the placenta of deer is a fairly common opportunistic pathogen of humans and animals. However, only one similar case of *C. parapsilosis* isolation from the placenta of an aborted cow has been described in the literature (Foley et al. 1987).

Cases of mycoses in reptiles also deserve attention. The yeast fungus *Candida duobushaemulonii* was isolated from the subcutaneous lesions of the blunt-nosed viper (*Macrovipera lebetinus*). *C. haemulonii* complex includes *C. haemulonii sensu stricto*, *C. duobushaemulonii*, and *C. haemulonii var. vulnera* species. *C. duobushaemulonii* species, judging by the available literature, has not been found in animals to date.

Table 1: Localization of viruses in organs and tissues

Viruses	Animal species			
	Sika deer	Red deer	Siberian roe deer	Moufflon
Rotavirus	small intestine lungs spleen	small intestine kidney lungs spleen	small intestine	lungs spleen trachea
Coronavirus	small intestine	-	small intestine	-
IRT	lungs lymph nodes	lungs trachea	spleen	spleen
VD	lymph nodes spleen trachea	-	-	lymph nodes spleen trachea
PI3	lungs lymph nodes trachea	-	-	lymph nodes trachea

For the first time in the Russian Federation, we have diagnosed a dangerous reptile pathogen *Ophidiomyces ophidiicola* isolated from little wart snakes *Acrochordus granulatus* (Fig. 3). Three snakes were delivered to the zoo from Jakarta, Indonesia. Shortly after arrival, multiple white vesicles were found on their bodies. All three snakes died within a month after arrival. Microscopy revealed fungal arthrospores and mycelium in the exudate from the lesions. *Ophidiomyces ophidiicola* was isolated from two of the three snakes and identified using ITS sequencing. This case demonstrates that endemic pathogenic fungi can easily enter the territory of our country.

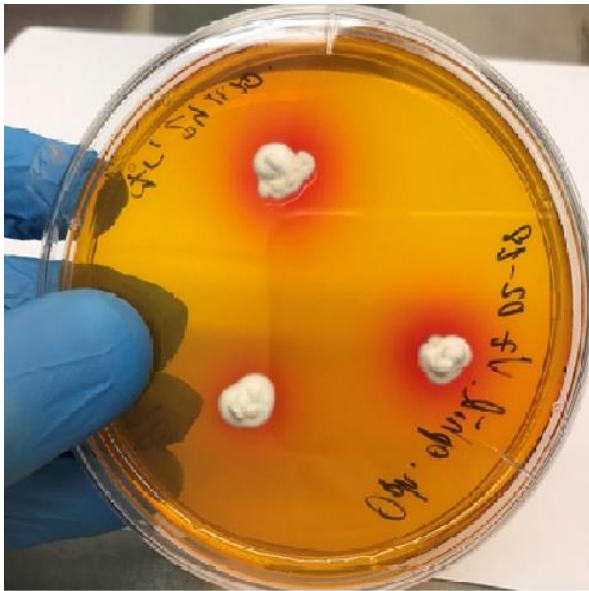


Fig. 3: *Ophidiomyces ophidiicola* isolated from a snake *Acrochordus granulatus* on DTM-Expert media, 7 days.

By metagenomic sequencing, 249 fungal taxa were found in the skin lesions of wild sables *Martes zibellina*, including keratinophilic fungi of *Onygenales* order, represented by *Trichophyton*, *Arthroderma*, *Chrysosporium*, *Malbranchea* and *Emmonsia* genera. During study, atypical cultures of dermatophytes were obtained, initially identified as *Arthroderma cuniculi*. However, further research together with czech colleagues (Moulikova et al. 2023) showed that we are dealing with a new species of fungus, for which the species name *Arthroderma zoogenum* sp. nov. was proposed (Fig. 4). *A. zoogenum* sp. nov. species was registered in Mycobank mycological nomenclature database (<https://www.mycobank.org/>) under the number MB 845987.

We have established for the first time that keratinophilic dermatophyte fungi, in particular *Arthroderma zoogenum*, are involved in the etiology of the skin disease affected up to 62% of fur-bearing sables in Siberia. Cultures of streptococci and staphylococci were

isolated from lungs, spleen, lymph nodes, liver, kidney, heart of siberian roe deer, sika and red deer, moufflons (n=16) with signs of respiratory disease in hunting farm (Fig. 5). The identification of isolates allowed them to be attributed to 7 species: *Staphylococcus aureus*, *Streptococcus parauberis*, *Streptococcus equi*, *Streptococcus suis*, *Staphylococcus cohnii*, *Streptococcus bovis*, *Staphylococcus aureus* spp. *anaerobicus* (Table 2). The cultures demonstrated hemolytic, serological and biochemical properties typical for these species.



Fig. 4: *Arthroderma zoogenum* sp. nov. isolated from sable *Martes zibellina* on Sabouraud media, 7 days.

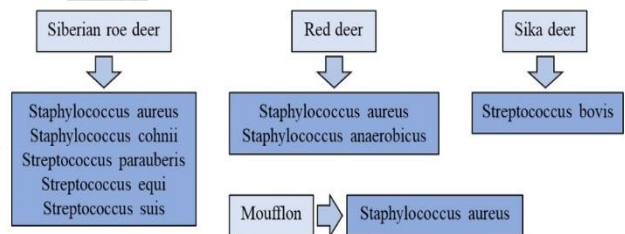


Fig. 5: Cocci isolated from wild artiodactyls.

The largest number of bacterial cultures were isolated from the lungs. In addition, *S. coxii* and *S. aureus* were isolated from the heart and liver of siberian roe deer respectively, *S. aureus* spp. *anaerobicus* – from the spleen of red deer (Table 2).

Most often, *S. aureus* cultures which were isolated from pathological material, gave positive results in glucose fermentation, presence of catalase, coagulase tests and negative in oxidase activity and sensitivity to bacitracin tests. The hemolytic activity of the isolated cultures led in most cases to the formation of a double α and β hemolysis zone. Cultures (up to 25%) had drug resistance to cefazolin, amoxicillin, cephotaxime, kanamycin, amoxiclav, streptomycin, amikacin, gatifloxacin, erythromycin,

Table 2: Bacteriological study results

Animal species	Localization of lesions				
	Lungs	Heart	Kidneys	Liver	Spleen
Siberian roe deer	<i>Staph. aureus</i> , <i>Str. parauberis</i> , <i>Str. equi</i> , <i>Str. suis</i>	<i>Staph. cohnii</i>	-	<i>Staph. aureus</i>	-
Sika deer	<i>Str. bovis</i>	-	-	-	-
Red deer	<i>Staph. aureus</i> spp. <i>aureus</i>	-	-	-	<i>Staph. aureus</i> spp. <i>aureus</i> ,
Moufflon	<i>Staph. aureus</i> spp. <i>aureus</i>	-	-	-	-

Table 3: *Staphylococcus aureus* susceptibility to antibiotics

Resistance to (up to 25% cultures)	The zones of inhibition, mm or less	Sensitive to	The zones of inhibition, mm or more
amoxicillin, penicillin, amoxiclav, gatifloxacin, ceftazidime, cefpirome	19	imipenem	13
cefazolin, amikacin, cephaclor, cephotaxime	14	trimethoprim	10
kanamycin, erythromycin	13	tylosin	12
streptomycin, metronidazole	11	ceftriaxone	13
ampicillin, tetracycline	18	ciprofloxacin, enrofloxacin	15
cefaperazone, lincomycin, pristinamycin	15	-	-
chloramphenicol	20	-	-
neomycin, tobramycin, sulfadiazine, sulfamethizole	12	-	-
oxacillin, nafcillin, monomycin, amandomycin	10	-	-
rifampicin	16	-	-

cefapirazon, ampicillin, cefpiron, ceftazidime, cefaclor, chloramphenicol, penicillin, lincomycin, neomycin, tobramycin, oxacillin, tetracycline, rifampicin, nafcillin, monomycin, metronidazole, pristinamycin, amandomycin, sulfadiazine, sulfamethizole. The isolated cultures were sensitive to ciprofloxacin, enrofloxacin, tylosin, trimethoprim, ceftriaxone, and imipenem (Table 3). Thus, *Staph. aureus* cultures isolated from wild artiodactyls had multiple drug resistance, and the range of active drugs is relatively narrow.

Streptococcus suis isolated from roe deer is an important pathogen for pigs. It is also found in cows, horses, sheep, goats, dogs. This species belonged to serogroup D had typical biochemical properties, fermented inulin, raffinose, showed a mixed type of hemolysis. *S. bovis* isolated from the sika deer was assigned to serogroup D. It is often found in the digestive tract of cows, other ruminants, horses, sheep, pigs. *S. equi* was isolated from the lung of roe deer belonged to serogroup C, fermented salicin, showed β -hemolysis. It is usually found in horses. *S. parauberis* isolated from the affected lung, had negative belonging to serogroups. All isolated *Streptococcus* species did not have catalase, oxidase, coagulase activity and were resistant to bacitracin (0.04 units/disc). The culture of *S. cohnii* was isolated from the heart of roe deer. This microorganism is present on human skin, causes cystitis and urethritis. The isolation of atypical cocci species from wild artiodactyls may be the result of interspecific transmission.

The sporadic cases of disease and death of wild animals that we observed were associated with pathogenic cocci. It is possible that wild animals may be included in the epizootic process as a source and reservoir of *Staphylococcus* and *Streptococcus* identified in this work.

Conclusion

Our studies have demonstrated the susceptibility of wild animals to a wide range of viruses, fungi and bacteria. In some cases, there was a correlation between the clinical picture and the identified pathogen, in others, this relationship was not obvious. Our studies demonstrate a high occurrence of clinically significant fungi in wild and exotic animals. For the first time, *Trichosporon lactis* was isolated from pathological muscle tissue of mammals, and *Phoma herbarum* and *Beauveria bassiana* from the brain. Moreover, first detection of *Candida duobushaemulonii* in animals was reported.

Analysis of the data obtained indicates the ability of wild animals to serve as a reservoir for a variety of clinically significant pathogens of domesticated animals and humans. Basing on the obtained results, we have acquired an expanded understanding about the species diversity of microorganisms in the wild fauna of the Russian Federation. This problem requires further intensive study.

Author's Contribution: AM Gulyukin – chief of the project, general management AF Shulyak – sample collection, virological investigation GN Velichko – virological investigation, RS Ovchinnikov – mycological studies, manuscript editing AG Gaynullina - mycological studies DJ Gorokhov – PCR-analysis AV Gorbatov – bacteriological investigation TA Ishkova – immunological investigation VV Belimenko – manuscript compilation.

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