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Pathoanatomic Picture of Eimeriosis Combined with Bacterial Infection and Candidiasis in Rabbits

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Abstract

The present work aims to diagnose the causes of the death of 1.5-2 month-old rabbits kept in a personal subsidiary farm, with the study of pathomorphology, parasitological analysis of the contents of the caecum, and bacteriological examination of internal organs. The disease was characterized by a sporadic course among rabbits of 1.5-2 months of age from April to July. A striking clinical sign of the illness is gastric bloating, visualizing during a general examination. The death of the animal occurred 2-3 days after the manifestation of a clear clinical picture. The pathoanatomic autopsy showed flatulence and inflammation of the caecum and colon, hepatosis, lymphadenitis, acute dilation of the right lung, pulmonary edema. During the parasitological examination of the contents of the caecum, Eimeria oocysts were found. Bacteriological examination of internal organs demonstrated an association of S. saprophyticus microorganism with hemolytic properties and microscopic fungi of the genus Candida spp. The association of S.saprophyticus and Eimeria synergistic microorganisms can disrupt colonization resistance of the intestine, leading to intestinal dysbiosis in an organism with unformed immunity. Against the background of dysbiosis, Candida spp. yeast actively multiplies, affecting the course of the infectious process, resulting in an acute process and a combined form of infection. Thus, postmortem diagnosis of eimeriosis should be aimed at identifying the pathogen of eimeriosis and identifying associative microorganisms.

Keywords: Eimeriosis, S.epidermidi, Candida spp., Rabbits, Infection

Introduction

Mixed infections from two or more pathogens, including representatives of the order Eimeria, are the norm for many reservoir hosts, including sheep and rabbits. However, there are still few reports on the study of competitive or stimulating interactions between microorganisms of various genera and families with protozoa–causative agents of coccidiosis. Coccidiosis is a common disease caused by representatives of the Eimeria order. Most often, coccidiosis manifests itself in the form of diarrhea, slow weight gain, and sometimes death, which causes serious economic losses for agriculture around the world (Al-Neama *et al.*, 2021).

Very often, eimeriosis (coccidiosis) occurs in rabbits (Lutfullina *et al.*, 2016). Young rabbits often get sick at the age of 1.5-4 months. The mortality rate of infection reaches 75-100% (Tolokonnikov *et al.*, 2015; Bezrukova *et al.*, 2016). In rabbits, there are three main types of pathogens of eimeriosis: Eimeria stiedae localized in the bile ducts, E. perforans, and E. magna that affect the small intestine (Linovitskaya & Kontsevaya, 2020).

The intensity of the invasive process caused by eimeriosis is influenced by interactions with other pathogens. Mixed infections of Eimeria with toxin-producing strains of C. perfringens caused necrotic enteritis in chickens and other birds and mammals. Besides, with mixed infection of Eimeria spp. with Escherichia coli, the synergism of the pathological effect concerning the manifestation of intestinal lesions has been demonstrated (Bangoura & Daugschies, 2018). Thus, the presence of Eimeria can enhance the result of joint infection with bacterial pathogens, such as C. perfringens (contributing to necrotic enteritis) or Salmonella enterica serovar enteritidis or typhimurium (Arakawa *et al.*, 1981; Qin *et al.*, 1995; Moore, 2016).

The course of the disease, manifestation, and clinical signs largely depends on the type of causative agent of eimeriosis and the consortium of microorganisms causing the combined infection. The combined infection of C.perfringens and Eimeria significantly reduces the species diversity of the jejunum microbiota, with a simultaneous increase in the number of Clostridium sensu stricto, of the Escherichia, Shigella, and Weissella genera, and significantly reducing the populations of microorganisms of the Lactobacillus family. Monoinvasia by the causative agent of eimeriosis itself is not capable of causing dysbiotic phenomena (Macdonald *et al.*, 2019; Yang *et al.*, 2019).

Studies by Macdonald *et al.* showed that eimeriosis caused significant changes in the diversity of some microbial taxa in the caecum microbiome, which correlated with the most severe pathology of the caecum. With eimeriosis, the number of taxa belonging to the Enterobacteriaceae order increases, and the number of Bacillales and Lactobacillales taxa decreases (Macdonald *et al.*, 2017).

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In turn, the bacterial flora of the caecum plays a huge role in the development of coccidiosis. Infection with coccidia stimulates the growth of opportunistic microorganisms, for example, C. perfringens, E. coli, Bacteroides spp., Candida albicans, and reduces the amount of Lactobacillus. In turn, associations of these microorganisms provoke the development of coccidiosis of the caecum in chickens (Mamom & Chawanit, 2010). The influence of co-infectors on the course of eimeriosis is expressed in the infectious process spread dynamics in the host body. The protozoa Eimeria consortium enhances Clostridium perfringens and Salmonella typhimurium in experimentally infected animals because increased mucus secretion and bleeding caused by eimeriosis serve as a source of nutrients and iron to support the growth of opportunistic microorganisms (Al-Neama *et al.*, 2021).

It has been observed that candidiasis can be associated with coccidiosis. C.rugosa was isolated from young turkeys with concomitant chronic coccidiosis. Laboratory studies after a pathoanatomic autopsy showed excessive yeast growth in the digestive tract with ulcerative lesions of the stomach (Vieira & Coutinho, 2009). Candida albicans are present in healthy animals' and birds' digestive, urinary, and reproductive tracts (Mancianti *et al.*, 2002). Candida often causes gastroenteropathy, acting as a primary or secondary infection. Clinical signs of candidiasis depend on the infection localization. The intestines are most often affected in young animals and birds, accompanied by constipation, bloating, depression, weight loss, and growth disorders. The degree of infection depends on the balance between the virulence of the microorganism and the host's defense mechanisms. There is evidence of the presence of highly pathogenic strains of Candida that cause infections in healthy people. Many microorganisms produce hydrolytic enzymes that enhance the pathogenicity of Candida, facilitating its penetration into tissues (Vieira & Coutinho, 2009). Research by Lesmana *et al.* indicated that pathogenic E.coli and Candida albicans were the most common microorganisms and parasites found in chronic infectious diarrhea (Lesmana *et al.*, 2004).

Pseudomonas aeruginosa, Acinetobacter baumannii, and Staphylococcus epidermidis usually coexist with C. albicans (Shirtliff *et al.*, 2009). Such polymicrobial infections cause several infectious syndromes, with the formation of mixed biofilms from various types of microorganisms formed due to the coagulation of C. albicans and several pathogenic bacteria on the surface of the animal and human body biotopes, which are considered important for the development of infectious diseases (Harriott & Noverr, 2011). Such fungal-bacterial interactions can be antagonistic, synergistic, commensal, or symbiotic and affect physical and physiological characteristics, such as mutual morphology, behavior, and survival, including reaction to antimicrobial agents (Frey-Klett *et al.*, 2011).

For example, the Staphyloccocus spp. microorganisms can create agglomerates with hyphae of C. albicans yeast cells, and their bacterial extracellular polymers can protect C. albicans from antifungal agents (Adam *et al.*, 2002; Wang *et al.*, 2014).

Materials and Methods

An autopsy was performed on rabbits that had died due to the disease during the first hour after the animal's death to study the pathological and morphological changes in the internal organs. A total of five animals were examined. A pathoanatomic autopsy was carried out according to the standard method of the autopsy of small animals, with the extraction of a single organ complex.

Definition of eimeria. The contents of the caecum were suspended in a mortar in 20 ml of water, filtered, the filtrate was centrifuged. A saturated solution of sodium chloride was added to the precipitate and centrifuged. Three drops were removed from the surface film with a bacteriological loop, transferred to a slide, covered with a cover, and examined in the darkened field of view of a microscope at a magnification of 140-280 times.

Bacteriological examination. After the autopsy, the seeding material was selected with a sterile Pasteur pipette, seeding into test tubes with meat-blanket broth. Tubes with meat-peptone broth were incubated at 37^0C for 18-24 hours. After incubation, sowing was carried out on agarized dense nutrient media: Endo medium, Ploskirev's agar, bismuth-sulfite agar, yolk-salt agar, blood agar, Sabouraud's agar. The cultures were incubated at 37^0C for 24-48 h, and those sown on Sabouraud's medium at 30^0C for up to 5 days. After incubation, the morphology and cultural properties of microorganisms were studied according to standard methods.

Results and Discussion

The age of the animals was about 60 days, hybrids of Burgundy rabbits and English Spot rabbits. The animals were kept in the rabbit house in cages, one animal per cage. Antiepizootic preventive measures were not carried out. No treatment was applied.

Clinical examination showed progressive deterioration of the general condition: depression, loss of appetite, diarrhea. The rabbits were emaciated, with a bloated belly and a dull, disheveled coat. The course of the disease was acute. After the manifestation of vivid clinical symptoms, death occurred on the 3rd/4th day. Mortality of animals with similar clinical manifestations was registered earlier and characterized by sporadic epizootic course from April to August. Young rabbits fell ill after weaning from the mother at the age of 1.5-2 months.

The general condition of the corpses was characterized by dehydration and general muscular atrophy. The visible mucous membranes were anemic.

The gastrointestinal tract was in a state of acute catarrhal inflammation of the caecum and colon. The colon was enlarged in volume, with pronounced flatulence. The contents of the intestine were a grayish-red semi-liquid mass. The blood vessels of the mesentery of the large intestine were filled with blood.

a)

b)

Figure 1. Pathoanatomic Changes in Eimeriosis, Combined with Bacterial Infection and Candidiasis

The liver was enlarged in volume; the edges were rounded, burgundy-red in color, the parenchyma was bulging on the incision, the organ outline was smoothed, with multiple dotted and small spotty hemorrhages under the capsule and in the parenchyma. Mesenteric lymph nodes were in a state of inflammatory hyperemia with spot hemorrhages on the surface. A clear bloody liquid flowed down on the incision. The heart was in a state of acute expansion of the right department. The lungs were enlarged in volume, apneumatic, dark red with a bluish tinge, smooth from the surface and on the incision, a dent remained when pressed, bloody foamy liquid flowed from the incision surface, pieces of the lung floated heavily in the water **(Figure 1)**.

Microscopic examination of preparations from the caecum contents demonstrated immature and sporulated oocysts of eimeria **(Figure 2)**.

Figure 2. Eimeria Oocysts Magnified 280 Times

As a result of a bacteriological examination, gram-positive microorganisms, such as S.epidermidis, S.saprophyticus, E.faecalis, and gram-negative microorganisms, such as E.coli, E.agglomerans, P.maltophiliae, Alcaligenes spp., and the yeast of the Candida spp. Genus were isolated from the internal organs of rabbits (gallbladder, lungs, heart, liver, brain, kidney).

Pure cultures of Candida spp. and S.saprophyticus (100%) with hemolytic properties were isolated from all samples from five heads of rabbits. The specific gravity of S.epidermidis was 7%, E.coli 6.6%, E.agglomerans 33.3%, E.faecalis 30%. Alcaligenes spp., P. Maltophiliae (3.3% each) were isolated in one animal **(Table 1, Figure 3)**.

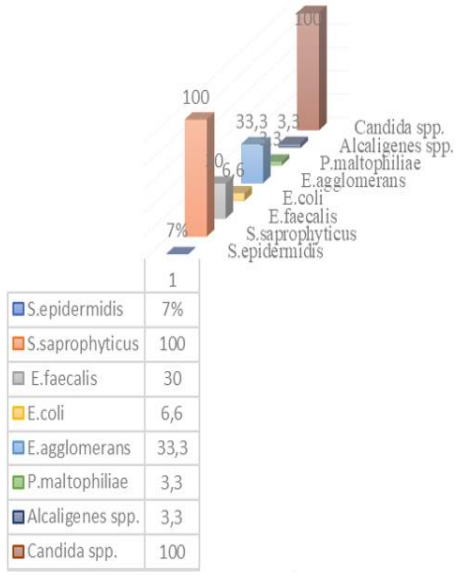

Figure 3. The Specific Gravity of Microorganisms from Pathological Material from rabbits.

Table 1. Microorganisms Isolated from the Internal Organs of Baby Rabbits

Item No.	Microorganisms isolated from internal organs					
	Liver	**Heart**	**Kidneys**	**Lungs**	**Brain**	**Gallbladder**
1.	S.saprophyticus; Candida spp.; E.agglomerans	S.saprophyticus; Candida spp.; E.agglomerans	S.saprophyticus; Candida spp.; P.maltophiliae; E.coli	S.saprophyticus; Candida spp.; E.agglomerans; E.faecalis; S.epidermidis	S.saprophyticus; Candida spp.; E.agglomerans; S.epidermidis	S.saprophyticus; Candida spp.; Alcaligenes spp.; E.coli; E.faecalis
2.	S.saprophyticus; Candida spp.; E.faecalis	S.saprophyticus; Candida spp.; E.agglomerans E.faecalis	S.saprophyticus; Candida spp.	S.saprophyticus; Candida spp.	S.saprophyticus; Candida spp.; E.agglomerans; E.faecalis	S.saprophyticus; Candida spp.
3.	S.saprophyticus; Candida spp.	S.saprophyticus; Candida spp.	S.saprophyticus; Candida spp.	S.saprophyticus; Candida spp.	S.saprophyticus; Candida spp.; E.faecalis	S.saprophyticus; Candida spp.
4.	S.saprophyticus; Candida spp.; E.faecalis; E.agglomerans;	S.saprophyticus; Candida spp.	S.saprophyticus; Candida spp.; E.agglomerans; E.faecalis	S.saprophyticus; Candida spp.	S.saprophyticus; Candida spp.	S.saprophyticus; Candida spp.; E.agglomerans;
5.	S.saprophyticus; Candida spp.	S.saprophyticus; Candida spp.	S.saprophyticus; Candida spp.; E.agglomerans; E.faecalis	S.saprophyticus; Candida spp.	S.saprophyticus; Candida spp.	S.saprophyticus; Candida spp.

The presence of eimeria in the bodies of the studied dead animals is justified because this group had not been subjected to periodic preventive treatments against eimeriosis.

Due to the associated course of infection, there is no species-specific picture characteristic of mono-infestation of eimeriosis, when extensive damage to a certain organ is observed, to the cells of which the found type of eimeria has a tropism. Against the background of an immature immune system in the body of baby rabbits, changes occurred that led to the reproduction of hemolytic S.saprophyticus, which indicates the potential of this microorganism to cause an infectious process. Against the background of the addition of Staphylococcus saprophyticus with hemolytic properties, the Candida spp. microorganism, which is a companion of mucosal dysbiosis, has actively developed.

In combination with parasitosis of eimeria, the associated infection caused persistent disorders in the body of various organs and systems characterized by acute enteritis, hepatosis, acute serous lymphadenitis, acute dilatation of the right heart department, lungs in a state of congestive hyperemia, and edema. The death of the animals occurred as a result of paralysis of the respiratory center with asphyxia due to pulmonary edema, as indicated by the expansion of the right heart.

Conclusion

In the postmortem diagnosis of eimeriosis, it is advisable to examine animal organs for secondary bacterial flora, including microscopic yeast Candida spp. The clinical presentation, the outcome of the disease, and pathomorphology largely depend on microorganisms' primary or secondary associations. In case of infection of rabbits with parasites such as Eimeria, pathogenic Staphylococcus saprophyticus, and Candida spp., a combined form of eimeriosis is observed with the involvement of several organs in the pathological process (intestines, liver, heart, lungs).

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