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GENITAL MYCOSIS IN MAIL DOGS

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ABSTRACT

As a result of microscopic and microbiological tests on semen from two dogs with signs of infertility necrospermia was established and fungi of the genus Fusarium were isolated. When develop in food and feed they emit toxins that cause mycotoxicosis in animals and birds after oral intake. Zearalenone, one of mycotoxins of these mushrooms is known as the cause of the atrophy of the gonads, spermatogenesis disorders, and infertility. This, however, is the first release for the isolation of fungi from the seminal fluid and setting them as the cause of genital mycosis.

Key words: infertility, Fusarium sp., genital mycosis, dogs.

Introduction

It is a well-known fact that for the successful application of various reproductive techniques, the good reproductive health of the breeding animals is of particular importance. In this connection, the quality of the ejaculates is crucial to obtaining optimum results in animal reproduction. There are, however, a number of cases in the practice, in which the quality of the semen does not meet the requirements for an optimal reproduction process. Possible causes of this are abnormal temperature regulation of testicles, trauma, haematocele, hydrocele, inflammatory process in testicles or epididimis, prolonged systemic disease, obesity (increased scrotal fat), prostatitis, brucellosis, medications, autoantibodies to sperm and others (Ticer, 1965; Johnston, 2000).

In this regard, the aim of the present study was to determine the cause of infertility in male dogs, a Middle Asian shepherd breed.

Materials and Methods

Animals under study. The research was carried out on materials from two male dogs aged 7 and 8 years of Middle Asian shepherd breed. They are grown together under the same living and eating conditions, in the yard of a house with farm animals. Throughout all period of the study, the dogs were in good general condition, without signs of infectious or non-infectious pathology. However, in attempts to fertilize female dogs infertility was established.

Clinical materials. Seminal fluid samples from both dogs were examined. They were obtained in sterile vials twice with an interval of one month. Seed fluid analysis was done on a computer sperm analyzer Nikon Eclypse 200.

Microscopic studies of native materials and after staining using the classical methods of Gram and Romanowsky-Giemsa were performed under an immersion at magnification of 1200 x.

Nutrient media. Selective nutrient media were used for isolation and quantification of the microorganisms (Antisel – Sharlau Chemie S. A., Spain): agar of Mueller Hinton, Eosin Methylene Blue agar for *E. coli* and the Gram-negative aerobic bacteria, Cetrimide agar for bacteria of the genus *Pseudomonas*, Chapman Stone agar for those of the genus *Staphylococcus*, Sabouraud agar

for fungi, selective agar for enterococci, differentiation liquid media (for indole determination, nitrate degradation, methyl red and Voges-Proskauer reactions) selective agar for *Clostridium perfringens* (Merck, UK), blood agar and broth of Tarocchi for obligate anaerobes (BUL BIO NCIPD Ltd. - Bulgaria).

For isolation of microorganisms, cultures from the samples were made in the elective and selective nutrient media for bacteria of different groups, as well as for fungi. They were cultured at 37° C and 28° C for 24 to 72 hours under aerobic conditions.

The taxonomic identification of the isolated bacteria was performed by microscopic examination of their morphology, reading the cultural features and biochemical properties using differentiating liquid media and additional tests for oxidase, hydrogen sulphide and others with reagents from Antisel (Sharlau Chemie S. A., Spain). The isolation and identification of the bacteria has been carried out in accordance with the Bergey International Identifier (Holt et al., 1994), and of the fungi was made according to Murray et al. (2003).

Determination of the sensitivity of isolated bacteria to antimicrobial means was carried out by the classic agar-gel diffusion method of Bauer et al. (1966). Standard antibiotic discs (BULBIO – NCIPD Ltd. – Sofia) and such prepared by us were used after inoculation of bacterial suspension in exponential growth phase with a concentration of 2.10^6 cells/ml on blood agar. Incubation was performed at 37°C for 24 hours. The results were interpreted in a three-tier system of Bauer et al. (1966) after measuring the diameters of inhibitory zones in mm.

Results

Microscopic studies. The results of the spermiogram of the materials from the studied dogs are presented in Table 1 and Fig. 1.

Table 1: CASA analysis of semen of dogs (Nikon Eclypse 200)

progression	Total	Percentage (%)	CONCENTRATION millions per ml	CONCENTRATION in total ejaculated
Static	101	99,0%	113,2	226,3
Non-progressive motile	1	1,0%	1,1	2,2
Progressive motile	0	0,0%	0,0	0,0
	102	100,0%	114,3	228,6
			(≥ 20 mill/ml)	(≥ 40 mill/total)

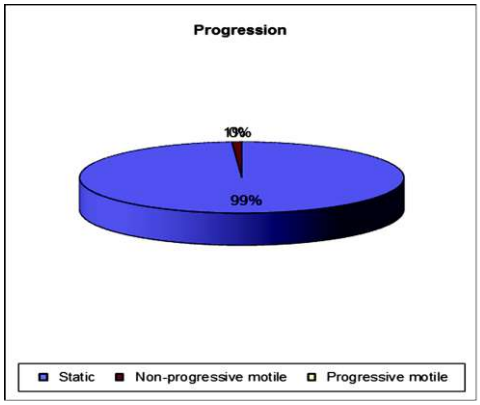

Figure 1: CASA analysis of semen of dogs (Nikon Eclypse 200)

As evidenced by the data of semen analysis, 99% of the sperm were non motile and only 1% showed signs of movement, in that not progressive but passive, ie. they were also not alive. Overall, no significant changes in sperm cells morphology were observed.

In the microscopic studies of the materials, conidia of fungi with morphology of representatives of the genus *Fusarium* were observed. They were large (about 8 μm) and pear-shaped. In examining the samples taken initially, bacteria were not found in the microscopic preparations. However, in seminal fluid samples obtained from both dogs after a one month period, except fungal conidia with morphology of *Fusarium* genus, Gram-negative rod-shaped bacteria were also observed.

***Cultural studies*.** The results of the cultural studies of both initially taken seminal samples are presented in Figure 2. Colonies of fungi with morphology characteristic of the genus *Fusarium* are seen. They developed after cultivation for 10 days and were large, mossy, oval or irregular in shape, with white to pink color. Data from colony and conidia morphology as well as growth indicators give reason to refer these to the species *Fusarium sporotrichella*. From these samples there were no isolated bacteria on the used elective and selective nutrient media.

Figure 2: Colonies of fungi of genus *Fusarium* on Saburough agar 10 days after application of semen samples of two dogs.

In the culture studies of semen samples obtained from the two patients after a one-month period, fungi of the same genus were isolated and determined by the morphology of conidia and colonies such as *Fusarium sporotrichella* (Figure 3a). From the same samples, Gram-negative facultative anaerobic bacteria were isolated, whose colonies can be seen in Figure 3b. According to the culture and biochemical indicators, they were defined as *Enterobacter agglomerans*. At the same time and Gram-negative obligate anaerobic bacteria with morphology characteristic of the *Dichelobacter* genus were isolated from both samples.

a b

Figure 3: Colonies of fungi of genus *Fusarium* on Saburough agar 9 days after application of semen samples of one of the dogs (a) and colonies of *Enterobacter agglomerans* on Mueller-Hinton agar of the same sample.

Sensitivity to antimicrobial means. The results of the *in vitro* tests to determine the sensitivity of the isolated *Enterobacter agglomerans* to antimicrobials from different groups are presented in Table 2.

Table 2: Sensitivity of the isolated bacteria Enterobacter agglomerans to antimicrobial means in vitro

Antimicrobial mean	Disc content (μg)	Sensitivity of the strains	
		P 1	P 2
Thiamphenicol	30	R	R
Tetracycline	30	R	R
Lincomycin	15	R	R
Oxacillin	1	R	R
Amoxycillin+Clavulanic acid	10	R	R
Penicillin	10	R	R
Cefuroxime	30	S	S
Cefotaxime	30	S	S
Novobiocin	30	S	S
Gentamicin	10	S	S
Amikacin	10	S	S
Enrofloxacin	5	S	S
Ciprofloxacin	5	S	S
Sulfamethoxazole+Trimethoprim	23,75/1,25	S	S

The data in the table shows the multiple resistance to antibiotics of the two isolated strains of *E. agglomerans.* It covers some broad spectrum products such as tetracyclines and amphenicols as well as those from the penicillin group, including amoxicillin in combination with clavulanic acid (β-lactamase inhibitor). The strains were sensitive to aminoglycosides, quinolones and potentiated sulfonamides.

Treatment. It was performed with metronidazole in combination with quinolone (enrofloxacin orally at a dose of 10 mg / kg with food every 12 or 24 h.

After administration of this therapy for three weeks a good effect was achieved. This is evident from the results of the spermogram of the materials from the examined dogs after the treatment, which are presented in Table 3 and Fig. 4. Data show that 24% of the sperm were mobile after completion of therapy.

Table 3: CASA analysis of semen of dogs after the applied antimicrobial therapy (Nikon Eclypse 200)

	Total	Percentage	CONCENTRATION	
progression		(%)	millions per ml	in total ejaculated
Static	3174	74,1%	389,2	389,2
Non-progressive motile	1036	24,2%	127,0	127,0
Progressive motile	76	1,8%	9,3	9,3
	4286	100,0%	525,5	525,5

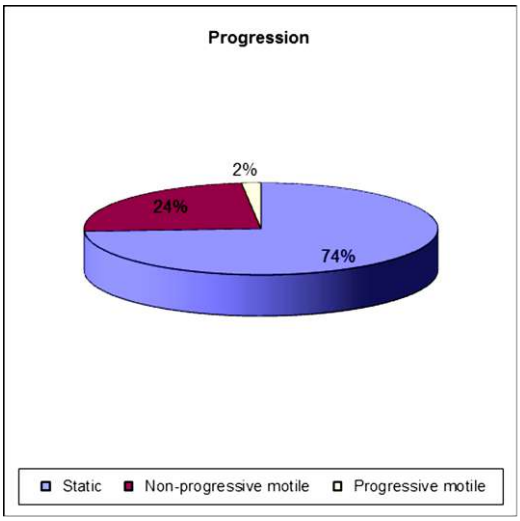

Figure 4: CASA analysis of semen of dogs after the administered treatment with antimicrobial means (Nikon Eclypse 200).

Discussion

The data from the performed studies show mycosis of the genital system of the studied patients and a following secondary bacterial infection. It is mixed with the participation of conditionally pathogenic Gram-negative aerobic and anaerobic bacteria. Most likely, the secondary infection was provoked by the fungi of the genus *Fusarium*, found in the seminal fluid. In animals and birds, the mycotoxins, and predominantly zearalenone, emitted by these fungi, after oral ingestion in the body, cause gonadal atrophy in both sexes with signs of excitability, false oestrus and infertility (Popova, 2016). However, there is insufficient research on their role as causative agents of mycoses. The isolation of *Fusarium sporotrichella* from such material is an indication for such a diagnosis. There are no literary data for mycoses of the genital system. This is the first report for isolation such fungi from semen, and twice, of two patients living under the same zoo-hygienic conditions and nutrition. Predisposing factors are intake of food with spores of molds and mycotoxins, as well as living in a damp premises and poor hygiene. The dogs studied were grown under similar conditions.

The use of quinolone was chosen in accordance with the antibiotic test results for *Enterobacter agglomerans*. Because the two isolated strains exhibited sensitivity to quinolones or aminoglycosides, but these are not active against obligate anaerobes, this therapy was supplemented with metronidazole directed against the isolated strict anaerobes of the genus *Dichelobacter*, as well as against the fungus *F. sporotrichella*. The established in vitro multiple resistance of the isolated *E. agglomerans* to antibiotics makes an impression, particularly to those of the penicillin group and to some broad-spectrum such as tetracycline and amphenicols. Such resistance is increasingly common among clinical isolates today. Even amoxicillin in combination with β-lactamase inhibitor (clavulanic acid) did not show effect *in vitro*. The uniform results of the antibiotic tests are an indicator of the role of uniform conditions of breeding and treatment of animals, as their close coexistence being a prerequisite for the exchange of microorganisms, including resistant antibiotics.

Good results from applied combination therapy over three weeks have shown that infertility caused by a combined genital infection involving fungi and bacteria can be treated successfully.

However, the prophylaxis it is of great importance, in which the role of providing good zoo-hygienic conditions and quality food has a leading significance.

Conclusions

Genital mycosis was established in dogs with signs of infertility. Fungi of the species *Fusarium sporotrichella* were isolated from the seminal fluid of the patients. Dogs breeding and feeding conditions may be a factor in the development of such a fungal mycosis resulting in infertility.

The development of fungi in the genital tract turns out to be a prerequisite for the development of a combined bacterial infection with the participation of conditionally pathogenic bacteria – a facultative anaerobe (*Enterobacter agglomerans*) and a strict anaerobe of the genus *Dishelobacter*.

Combined therapy for three weeks with metronidazole and enrofloxacin had a very good result – 24% mobile sperm.

References

1. Bauer, A. W., W. M. Kirby, J. C. Cherris, M. Truck. (1966). *Antibiotic susceptibility testing by a standardized single disk method*. The Am. J. of Clin. Pathol., 45–4, 493–496.
2. Holt, J. G., N. R. Krieg, P. H. A. Sneart, J. T. Staley, S. T. Williams. (1994). *Bergey's manual of determinative bacteriology*. Ninth edition, Williams & Wilkins.
3. Murray, P. R., E. J. Baron, J. H. Jorgensen, M. A. Pfaller, R. H. Yolken. (2003). *Mannual of Clinical Microbiology*. 8th Edition, Vol. 2, ASM Press, Washington, D. C., 1726–1760.
4. Popova, T. (2016). *Microbiology*. Publishing house at LTU, Sofia, 309–311.
5. Ticer, J. W. (1965). *Canine infertility associated with Pseudomona aeruginosa infection*. JAVMA, 146:720–722.
6. Johnston, S. D., K. Kamolpatana, M. V. Root-Kustritz, G. R. Johnston. (2000). *Prostatic disorders in the dog*. Animal Reproduction Science, 60–61: 405–415.

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RADIOLOGICAL STUDIES OF SECONDARY COMPLICATED SINUSITIS IN A RACING MARE – CASE REPORT

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ABSTRACT

The purpose of this research reflects the development process of secondary sinusitis in horses regarding the topographic-anatomical preconditions for unilateral involvement of the all six sinuses complex. The medical anamnesis and diagnostic imaging tests conducted previously in a veterinary clinic in Germany were used. The head of the mare was examined by X-ray radiography and computed tomography (CT) methods after its death. The following procedure included a treatment of the skull and the established osteolytic alterations have been compared by us with those obtained from the X-ray images and CT scans, as well as the applied CT slices and 3D reconstructions of the alive patient. This prominent clinical case reveals an opportunity for an interpretation of the expansion and complications of sinusitis in horses with an emphasis on the anatomical characteristics of the sinuses, visualized by diagnostic imaging methods. Through this study we hope to contribute to the timely diagnosis and treatment of the paranasal sinuses inflammation in horses.

Key words: paranasal sinus system, secondary sinusitis, computed tomography, horse.

Introduction

The equine paranasal sinuses are an intricate area of interests. The horse head had six pairs of sinuses, three paranasal; the frontal, maxillary and sphenopalatine sinuses and three nasal; dorsal, middle and ventral conchal sinuses and all of these spaces communicate with each other and the nasal passage either directly or indirectly. Different sinus compartments communicate with each other, grossly creating a rostral and more caudal complex (Vlaminck, 2013). The rostral complex consists of the ventral conchal sinus which communicates with the rostral maxillary sinus over the infraorbital canal through the conchomaxillary opening. The caudal complex consists of the caudal maxillary sinus which broadly communicates with the conchofrontal sinus through the frontomaxillary opening. Over the infraorbital canal, the caudal maxillary sinus also communicates with the more medially located sphenopalatine sinus. Caudal maxillary sinus communicates with middle conchal sinus. Rostral and caudal maxillary sinuses communicate with the nose through separate narrow nasomaxillary openings into the middle meatus. This close communication with the nose renders these sinuses vulnerable for development of infectious problems.

The large size and complex anatomy of the sinuses can allow a pathologic process to be present for weeks or months before any external signs, such as facial swelling or nasal discharge were noticed by the owner or veterinarian. This can negatively affect the prognosis (Waguespack, 2011).

Disease processes that can develop in the sinuses include: ethmoid hematomas, cysts, neoplasia, and bacterial and fungal infections.

Clinical signs of any type of sinusitis usually include unilateral purulent nasal discharge, ipsilateral mandibular lymph node enlargement, and epiphora. Less common signs include facial swelling, exophthalmos, abnormal respiratory noises, head shaking, and exercise intolerance (Lane 1993; Tremaine & Dixon, 2001a).