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Original article

MICROBIOLOGICAL AND PARASITOLOGICAL INVESTIGATIONS IN RODENTS POSING SANITARY RISK IN ANIMAL FACILITIES

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Summary

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A total of 88 rodents – 22 Norway rats *(Rattus norvegicus),* 36 black rats *(Rattus rattus)* and 10 house mice *(Mus musculus),* were caught in different animal facilities and two zoos. Additionally, 10 albino rats and 10 laboratory mice from a specialised vivarium were studied. Samples of hair, intestinal tract and liver were examined microbiologically. Faecal samples from the three synanthropic rodent species were investigated for presence of helminth eggs, and diaphragms of carcasses – for presence of *Trichinella* larvae. Microbiological examination showed that synanthropic rodents carried enterotoxigaenic *Escherichia coli*, *Staphylococcus aureus, Staphylococcus epidermidis* strains and some bacteria such as *Klebsiella pneumoniae, Citrobacter freundi, Enterococcus faecalis*, *Proteus spp. Moraxella atlantae,* posing a potential risk for farm animal health. The detected eggs of parasites as *Aspiculuris tetraptera*, *Hymenolepis* spp., *Eimeria* spp., *Oxyiuridae* spp., were specific for synanthropic rodents.

Key words: animal facilities, rodents, sanitary risk

INTRODUCTION

In their habitat, murids spread microorganisms and parasites through both biological and mechanical routes – via their contaminated hair, nibbling on foodstuffs or food products or contaminating them directly with faeces and urine (Mesina & Campbell, 1975; Kesyakova & Korudzhijski, 1981; Gacheva *et al.*, 2000; Acha & Szyfres, 2001; Kesyakova *et al.*, 2013).

Animal farms are artificial ecosystems, where harmful rodents are unsolicited but due to the specific environmental conditions, their populations are often numerous and flourishing. For complete and substantiated evaluation of sanitary risk posed by rodents in a given facility, one should be aware not only about the size of the respective population, but also detection, identification and classification of microorganisms carried by rodents into pathogens and conditional pathogens is needed. Such investigations have been done by researchers in different countries (Mesina & Campbell, 1975; Acha & Szyfres, 2001; Francis, 2002), but in Bulgaria, such research has not been conducted for years (Kesyakova & Korudzhijski, 1981). At the same time, modern trends for health risk assessment require control "from the stable to the table". Therefore, the microbial pathogens and parasites carried by synanthropic rodent populations in animal facilities should be recognised.

In a previous study of ours, we investigated the pathogenic microorganisms and parasites in rodents, caught in a food-processing enterprise (Kesyakova *et al.*, 2013).

The aim of the present study was to investigate the prevalence of microorganisms and parasitic eggs among rodents inhabiting different animal facilities.

MATERIALS AND METHODS

Samples from synanthropic rodents caught in eleven animal premises were submitted to microbiological and parasitological examinations. The surveyed sites were as followed: cattle farm (Stara Zagora region), pig farm (Rousse region), rabbit farm (Stara Zagora region), 4 poultry farms (Chirpan, Stara Zagora and Nova Zagora regions), the city zoos in Sofia and Stara Zagora, an experimental farm for rearing of pigs, sheep, goats and poultry (Sofia region), and a vivarium for laboratory animals. A total of 88 rodents – 22 Norway rats *(Rattus norvegicus),* 36 black rats *(Rattus rattus),* 10 house mice *(Mus musculus),* 10 albino rats and 10 laboratory mice were studied. Samples of hair, intestinal tract and liver were examined microbiologically. Faecal samples from the three synanthropic rodent species were investigated for presence of helminth eggs, and diaphragms of carcasses – for presence of *Trichinella* larvae. All animal facilities were free from infectious diseases at the time of survey.

The samples were analysed in the National Diagnostic and Research Veterinary Medical Institute, Bulgarian Food Agency – Sofia. Microbiological tests were run according to Bulgarian state standards as ISO 6579; 6888-02; 7251-05; 11133-1 etc. (Anonymous, 2004).

Bacteriological studies for isolation and identification of *Staphylococcus* spp*., Streptococcus* spp. and the Enterobacteriaceae family were done as per Bergey's Manual of Determinative Bacteriology (Holt *et al.* 1994). *E. coli* belonging to the ETEC pathovar were detected using agglutination kit K 88 (F 4) (Fimbrex, Vetwey, Weybridge, UK); Anti-Coli F 4 (K88) test serum (Sifin, GmbH, Berlin); *Escherichia coli* antigen test kit K-99 Pilitest, VMRD, US and Anti-Coli F 5 (K99) (Sifin, GmbH, Berlin). The biochemical identification was done via KBO10 Hi *E. coli* Identification Kit and the MICRONAUT E system (Merlin Diagnostika GmbH). Samples were tested for *Salmonella* spp. according to BSS EN ISO 6579/A1, Amendment 1, Appendix D (Serbezov, 1965; Bijlsma *et al.*, 1984; Masalski *et al.* 1995; Francis, 2002; Bischoff *et al.*, 2005).

The parasitological studies for parasitic eggs were performed by the Fülleborn's method. Tests of diaphragms from rats and mice for *Trichinella* larvae were done by the tissue compression method.

Table 1. Microbiological findings in rodents inhabiting different types of animal premises

Rodent species	Microbiological findings			Animal premise
	Skin surface	Intestinal tract	Liver	
Rattus norvegicus	*S. aureus* *Bacillus* spp.	*S. aureus* *Bacillus* spp. *S. aureus* (β-haemolytic) *Proteus* spp.	negative	Pig farm
Rattus norvegicus	*S. aureus* (β-haemolytic) *Proteus* spp.	*S. aureus* *E. coli* F5 (+)	*E. coli* F5 (+)	Cattle farm
Rattus rattus	*Moraxella atlantae*	*E. coli* F5 (+)	*Staphylococcus* spp.	
Rattus norvegicus	*S. aureus*	*Staphylococcus* spp. (non-haemolytic) *Proteus* spp.	*E. coli* F5 (+)	Poultry farms
Rattus rattus	*Staphylococcus* spp.	*Staphylococcus* spp.	*Staphylococcus* spp.	
Rattus rattus	*S. epidermidis* *Str. pyogenes* *S. saprophytycus*	*E. coli* F4 (+) *E. coli* F5 (+) *Klebsiella pneumoniae* *Enterococcus faecalis*	*E. coli* F4 (+) *E. coli* F5 (+)	Rabbit farm
Rattus norvegicus	*Streptococcus* spp. (α-haemolytic) *S. epidermidis* *Klebsiella pneumoniae* *S. saprophyticus*	*E. coli* F5 (+) *Citrobacter freundi* *Enterobacter* spp. *Klebsiella pneumoniae* *Proteus vulgaris* *E. coli* F4 (+) *Enterococcus faecalis*	*E. coli* F5 (+) *E. coli* F4 (+)	Experimental farm
Rattus rattus newborn	*Staphylococcus* spp.) (non-haemolytic)			City zoo
Mus musculus	*Staphylococcus* spp. (α-haemolytic)	*Staphylococcus* spp. (α-haemolytic)		City zoo

RESULTS

The data from microbiological tests of rodents caught in the different animal premises are presented in Table 1. The results indicated that studied synanthropic rodents carried mainly conditionally pathogenic microorganisms, frequently encountered in the environment.

The results from parasitological tests of rodent intestinal content and faeces are listed in Table 2. None of studied diaphragm samples showed larvae of *Trichinella*.

DISCUSSION

The data from the study confirmed that the presence of enterotoxigaenic *E. coli* as well as of haemolytic *Staphylococcus* spp. in harmful rodent populations should not be underestimated (Mesina & Campbell, 1975; Acha & Szyfres, 2001; Francis, 2002; Shepard *et al.,* 2012; Himsworth *et al.*, 2014). The isolation of the so-called super bacterium *Klebsiella pneumoniae* is another alarming sign that rodents carry microbial pathogens with potential high antimicrobial resistance. The passage of such microorganisms though the susceptible rodent population could lead to increased virulence and hence, higher risk for the health of productive animal and men. Some authors (Van De Giessen *et al.*, 2009; Himsworth *et al.*, 2014) reported the presence of methicillin-resistant *S. aureus* (MRSA) in Norway rats, including strains causing disease in men and farm animals. They affirmed rodents as an important source of infection, being a natural reservoir of MRSA over a prolonged period of time. Synanthropic rodents, which are the most important risk factors for contamination of animal foodstuffs, are the link in infection spread between men and animals (Himsworth *et al.*, 2014; Van De Giessen *et al.,* 2009).

The detection of *Moraxella atlantae* – a bacterium carried mainly by flies – on the skin of rats confirmed once again the indirect involvement of rodents in spread mechanisms, conservation and contamination of animal facilities with this microbial pathogen. The presence of these bacteria

Table 2. Parasitological findings in the gastrointestinal tract of rodents inhabiting different types of animal premises

Rodent species	Parasitological findings	Animal premise
Rattus norvegicus	negative	Pig farm
Rattus rattus	negative	
Rattus norvegicus	*Aspiculuris tetraptera*	Cattle farm
	Hymenolepis spp.	
	Eimeria spp.	
Rattus rattus	*Hymenolepis* spp.	
Rattus norvegicus	*Eimeria* spp.	Poultry farms
Rattus rattus	*Aspiculuris tetraptera*	
	Eimeria spp.	
Rattus rattus	*Hymenolepis* spp.	Rabbit farm
Rattus rattus – albino	*Oxyiuridae* spp.	Vivarium for laboratory rodents
Mus musculus – albino	*Oxyiuridae* spp.	

at the farms, sustained by the continuous contamination by rodents incurs not only health risks, but creates conditions for increased microbial load of the organism of animals. Thus the increased energy expenditure for the constant control of the microorganisms results in reduced yields and poorer economic results.

Data from parasitological tests showed that over the experimental period, rodents had no epizootological importance in none of surveyed animal facilities as parasitic diseases were concerned. The detected parasitic eggs are a specific finding for synanthropic rodents, proving once more that their populations should be kept under control through eradication.

CONCLUSIONS

The presence of rodents in animal facilities poses epizootological risks for farmed animals. The relatively most important risk is posed by rodents, inhabiting farms where multiple animal species are simultaneously housed. Norway rats *(Rattus norvegicus)* carried more microbial pathogen species and therefore, their presence at farms was related to higher epizootiological risk.

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