Research Article

www.ijrap.net

THE RESULTS OF THE IDENTIFICATION OF BACTERIA ISOLATED FROM THE DIGESTIVE TRACT OF BIRDS MELOPSITTACUS UNDULATUS OF HOME CONTENT

Laishevtcev Aleksey [1]*, Pimenov Nikolai [2], Tuhfatova Regina [2]

[1]All-Russian Research Institute of Experimental Veterinary Medicine named after Y.R. Kovalenko, Moscow, Russia

[2]Federal State Budgetary Educational Institution of Higher Education «Moscow state Academy of Veterinary Medicine and Biotechnology – MVA by K.I. Skryabin», Moscow, Russia

Received on: 20/06/16 Revised on: 19/07/16 Accepted on: 07/08/16

***Corresponding author**
E-mail: a-laishevtsev@bk.ru

DOI: 10.7897/2277-4343.074173

ABSTRACT

The bacteria of families Enterobacteriaceae, Staphylococcaceae and Streptococcaceae are the main representatives of the microflora of the digestive canal of budgerigars. The aim of the study is to determine the microbial profile of budgerigars contained in the conditions of Moscow and the Moscow region with the identification of pathogenic microorganisms. The material of the study was presented by clinical material obtained from the 286 specimens of budgerigars. The results of the determination of normal and pathogenic microflora of the digestive canal of clinically healthy budgerigars birds (*Melopsittacus undulatus*) contained in the conditions of the city of Moscow and the Moscow region are shown in article. The possibility of the bacterial carriage of saprophytic conditionally pathogenic microorganisms *Acinetobacter spp., Alcaligenes spp., Haemophilus spp., Corynebacterium spp., Klebsiella spp., Morganella osloensis, Pasteurella spp., Pseudomonas spp., Stenotrophomonas maltophilia*, which may pose a risk of human infection, is revealed.

KEY WORDS: Budgerigars, microbiocenosis, bacterial carriage.

INTRODUCTION

The content of decorative and exotic birds in the urban environment (in particular in the apartment conditions) today is not uncommon. At the same time the variety of these birds impresses with its species composition.

Being in close contact, people and birds are at risk of cross infection, there is the risk of transmitting an infectious agent both from person to bird, and vice versa [1,2]. At the same time, the majority of the representatives of enterobacterial and coccal microflora, which is the main component of the microbial profile of the digestive canal of birds, do not have any clearly expressed species specificity and selectivity in relation to the host [3]. It is quite difficult to find in literary sources a material about the state of the microflora of the digestive tract of decorative and exotic birds. That is why the scientific data, obtained during the study, will enrich the knowledge in the field of ornithology and sanitary safety [4,5].

The bacteria of families *Enterobacteriaceae, Staphylococcaceae* and *Streptococcaceae* are the main representatives of the microflora of the digestive canal of budgerigars [6, 7]. *Streptococcaceae* family is represented by the bacteria of genus *Enterococcus* and is found in the 69.44% of the specimens of birds, *Staphylococcaceae* family – by the bacteria of genus *Staphylococcus,* which is found in the 69.44% of the specimens of birds. *Enterobacteriaceae* family has the largest variety of genus representatives, among which Escherichia - 53.33%, Enterobacter - 38.33% and Proteus - 32.78% are most often indicated [8,9]. Moreover, a number of works shows the importance of the anaerobic representatives of normal microflora in exotic species of birds [10]. However, the given data do not characterize the pathogenicity of these microorganisms. In this regard, studies in this area have sufficient relevance.

The aim of the study is to determine the microbial profile of budgerigars contained in the conditions of Moscow and the Moscow region with the identification of pathogenic microorganisms.

MATERIALS AND METHODS

The material of the study was presented by clinical material obtained from the 286 specimens of budgerigars. Material was taken from clinically healthy birds that did not exhibit clinical signs of disease.

The following culture media were used for the research: Endo agar, Salmonella chromogenic medium cm1007, bismuth sulphite agar, beef-extract agar, beef-extract broth, Sabouraud agar, Salmonella Shigella agar, HiChrome agar, McConkey agar, citrate agar, xylose lysine deoxycholate agar (XLD agar), ONOZ Salmonella agar, Yersinia selective agar, blood agar, MRS agar. For the identification of cultures the following test systems were used: MICROBACT Staphylococcus 12S, MICROBACT 12E/A и 24E, STREPTO test 16. During the work, epizootic, microscopic, bacteriological, biological, serological, statistical research methods were used. Determination of the pathogenic properties of causative agent was carried out on SPF white mice weighing 16-18 g.

In the period from 2013 to the present time, the study of the structural composition of the bacterial and fungal microflora in the home decorative and exotic birds contained on the territory of Moscow and the Moscow region was held, including: Amazon parrot, African grey parrot, lovebird, crimson-bellied conure, rose-ringed parakeet, lorikeet, rosella, caique, eclectus parrot, Jardine's parrot, red-crowned parakeet, macaw, cockatoo, cockatiel and budgerigar. The total number of birds that were used in the study exceeded two thousand specimens.

Since the results have a significant amount, their analysis and structured conclusions cannot be given in a single article. In this regard, research results for each species of birds will be analyzed and shown separately. In this publication we will consider the composition of the bacterial and fungal microflora isolated from the pharynx and cloaca of the birds of genus *Melopsittacus*, species budgerigar - *Melopsittacus undulatus*. During the research, one smear from the mucosa of the pharynx and cloaca of each bird was taken on transport systems with Amies medium.

Methodology of the bacteriological examination

Sample was investigated within 72 hours after sampling. In this period of time bacteriological material was in a refrigerator with the temperature regime +2 +8 °C. For the isolation and identification of fungal microflora Sabouraud agar was used with the addition of 0.2 ml "Baytril" on 250 ml of medium to suppress growth of bacterial flora. After seeding, Petri dishes were placed in a thermostat with temperature 21 °C for a period of not less than 7 days, followed by daily growth control. Isolation of bacterial flora was carried out by enrichment for saving potential single colonies in the taken material on beef-extract broth. Cultivation on the beef-extract broth lasted 24 hours in the thermostat with a temperature 37 °C. After incubation, the obtained material was reseeded on elective and differential solid nutrient media. After the inoculation of nutrient media, the identification and typing of isolates was carried, based on their cultural, morphological, biochemical and antigenic properties.

Table 1: Results of the identification of bacteria isolated from the pharynx of budgerigars

№	Genus affiliation of bacteria	Number of isolates	Percentage of indication among birds*
1	*Acinetobacter spp.*	16	5,59
2	*Alcaligenes spp.*	8	2,80
3	*Bacillus spp.*	21	7,34
4	*Bordetella spp.*	2	0,70
5	*Capnocytophaga spp.*	1	0,35
6	*Citrobacter spp.*	6	2,10
7	*Corynebacterium spp.*	8	2,80
8	*Diplococcus spp.*	1	0,35
9	*Enterobacter spp.*	43	15,03
10	*Enterococcus spp.*	48	16,78
11	*Escherichia spp.*	19	6,64
12	*Haemophilus spp.*	4	1,40
13	*Klebsiella spp.*	13	4,55
14	*Lactobacillus spp.*	24	8,39
15	*Micrococcus spp.*	33	11,54
16	*Moraxella osloensis*	1	0,35
17	*Morganella spp.*	1	0,35
18	*Nocardia asteroides*	5	1,75
19	*Pasteurella spp.*	5	1,75
20	*Proteus spp.*	3	1,05
21	*Providencia spp.*	1	0,35
22	*Pseudomonas spp.*	12	4,20
23	*Sarcina spp.*	4	1,40
24	*Serratia spp.*	2	0,70
25	*Staphylococcus spp.*	131	45,80
26	*Stenotrophomonas maltophilia*	1	0,35
27	*Streptococcus spp.*	62	21,68
28	*Yersinia enterocolitica*	1	0,35
Total amount:		476 isolates	286 specimens

*Percentage of indication was calculated relative to the total number of birds collected for research

RESULTS

The total number of birds *Melopsittacus undulatus*, from which was obtained bacteriological material, was 286. During the bacteriological examination in most cases the associations of bacteria were isolated. During the analysis of the results the fact that the greatest generic diversity of bacteria was isolated from pharynx but not from cloaca was interesting. After the indication of bacteria, their further differentiation and identification were carried out till the genus affiliation. The results of the identification and the frequency of bacteria indication from the pharynx of budgerigars are shown in Table 1.

Bacteria of the genus *Staphylococcus spp.* from the pharynx of bird were the most frequent representative of microflora of bird pharynx. With a frequency of indication 131 case, this microorganism was isolated from 45.80% of the birds. The number of coagulase-positive isolates was 15: *Staphylococcus aureus* – 10 isolates, *Staphylococcus intermedius* – 5 isolates. In other cases, staphylococcus was coagulase-negative. Such an amount of staphylococcus indication suggests that this microorganism is the saprophytic microorganism and does not cause clinical symptoms, except coagulase-positive isolates.

The second most frequently isolated microorganisms - bacteria of the genus *Streptococcus* - 62 cases, isolated from 21.68% of birds. It should be noted that the number of the isolates of hemolytic streptococci was equal to 6.

Next places on indication frequency in the pharynx of birds are occupied by bacteria of the genera *Enterococcus spp*., *Enterobacter spp.* and *Micrococcus spp*.: 48 cases – 16,78%, 43 cases – 15,03% and 33 cases – 11,54% respectively. The next on the percentage of occurrence are the bacteria of genus Lactobacillus spp. 24 cases in 8,39% of birds, then Bacillus spp. – 21 isolates in 7,34% of birds, Escherichia spp. – 19 cases of indication in 6,64% of birds and Acinetobacter spp. – 16 cases

of isolation in 5,59% of birds. Bacteria of other genera have the indication frequency of less than 5%.

According to available literature data, microorganisms of genus *Bacillus spp.* (except *Bacillus anthracis*), *Enterobacter spp.*, *Enterococcus spp.*, *Escherichia spp.*, *Lactobacillus spp.*, *Micrococcus spp.*, *Proteus spp.*, *Staphylococcus spp.* (except coagulase-positive species), *Streptococcus spp.* (except hemolytic species) are the representatives of the normal microflora of the bird's body.

Potentially virulent representatives of microflora were used in pathogenicity studies. Determination of the pathogenic properties of isolates was conducted in a biological sample on white mice. Pathogenicity was determined for the following cultures: *Acinetobacter spp.* – 16 isolates, *Alcaligenes spp.* – 8 isolates, *Bordetella spp.* – 2 isolates, *Diplococcus spp.* – 1 isolate, *Capnocytophaga spp.* – 1 isolate, *Citrobacter spp.* – 6 isolates, *Corynebacterium spp.* – 8 isolates, *Klebsiella spp.* – 13 isolates, *Haemophilus spp.* – 4 isolates, *Moraxella osloensis* – 1 isolate, *Morganella spp.* – 1 isolate, *Nocardia asteroides* – 5 isolates, *Pasteurella spp.* – 5 isolates, *Providencia spp.* – 1 isolate, *Pseudomonas spp.* – 12 isolates, *Sarcina spp.* – 4 isolates, *Serratia spp.* – 2 isolates, *Stenotrophomonas maltophilia* – 1 isolate, *Yersinia enterocolitica* – 1 isolate.

For the preparation of bioassay, suspension was obtained from the daily culture of pathogen with a concentration of 1 bln. bacterial cells in 1 cm^3. The suspension was subcutaneously injected to 2 white mice for each isolate in a volume of 0.5 cm^3. Observing the animals was conducted within 7 days. Isolate was recognized as pathogenic after mice death during the observation period.

According to the research established that pathogenic properties have 2 of 16 *Acinetobacter spp.*, 6 of 8 cultures *Alcaligenes spp.*, 4 of 4 cultures *Haemophilus spp.*, 1 of 8 isolates *Corynebacterium spp.*, 6 of 13 isolates *Klebsiella spp.*, 1 isolate *Morganella osloensis*, 2 of 5 cultures *Pasteurella spp.*, 8 of 12 cultures *Pseudomonas spp.*, 1 isolate *Stenotrophomonas maltophilia*.

Cultures *Bordetella spp., Diplococcus spp., Capnocytophaga spp., Citrobacter spp., Moraxella osloensis, Nocardia asteroides, Providencia spp., Sarcina spp., Serratia spp., Yersinia enterocolitica* did not possessed pathogenic properties.

During analysis of the results of bacteriological examination of material from the cloaca of budgerigars we obtained the following results listed in Table 2.

Table 2: Results of the identification of bacteria isolated from the cloaca of budgerigars

№	Genus affiliation of bacteria	Number of isolates	Percentage of indication among birds*
1	*Acinetobacter spp.*	14	4,90
2	*Alcaligenes spp.*	6	2,10
3	*Bacillus spp.*	31	10,84
4	*Bordetella spp.*	1	0,35
5	*Corynebacterium spp.*	7	2,45
6	*Diplococcus spp.*	2	0,70
7	*Enterobacter spp.*	27	9,44
8	*Enterococcus spp.*	43	15,03
9	*Escherichia coli*	20	6,99
10	*Flavobacterium spp.*	2	0,70
11	*Hafnia alvei*	1	0,35
12	*Klebsiella spp.*	6	2,10
13	*Lactobacillus spp.*	194	67,83
14	*Micrococcus luteus*	23	8,04
15	*Nocardia asteroides*	5	1,75
16	*Pasteurella spp.*	2	0,70
17	*Proteus spp.*	2	0,70
18	*Providencia spp.*	1	0,35
19	*Pseudomonas spp.*	5	1,75
20	*Sarcina spp.*	4	1,40
21	*Staphylococcus spp.*	64	22,38
22	*Stenotrophomonas maltophilia*	1	0,35
23	*Streptococcus spp.*	40	13,99
Total amount:		501 isolates	286 specimens

*Percentage of indication was calculated relative to the total number of birds

Table 3: Results of the identification of the fungal microflora of budgerigars

Cloaca			Pharynx		
Genus	Number of isolates	%	Genus	Number of isolates	%
Aspergillus spp.	2	0,70	*Aspergillus spp.*	8	2,80
Candida spp.	17	5,94	*Alternaria spp.*	2	0,70
Malassezia spp.	2	0,70	*Candida spp.*	29	10,14
Mucor spp.	2	0,70	*Malassezia spp.*	3	1,05
Penicillium spp.	5	1,75	*Mucor spp.*	1	0,35
-	-		*Penicillium spp.*	16	5,59
Total:	28		Total:	59	

Analysis of the results shows that the predominant bacterial unit isolated from the cloaca of budgerigars is a genus *Lactobacillus spp.* with a frequency of indication 194 cases - 67.83%, respectively. The prevailing value of the lactic acid bacteria leads to the conclusion that this kind of microorganisms is an obligate intestinal representative of the studied species of birds, which is consistent with data on the symbiotic intestinal microflora [11,12].

The second place on the frequency of isolation among cloacal microorganisms belongs to the genus *Staphylococcus spp.* - 64 isolates was isolated from 22.38% of the birds. At the same time among 64 isolates from the cloaca 28 were coagulase-positive: *Staphylococcus aureus* – 17 isolates, *Staphylococcus intermedius* - 11 isolates. In other cases, staphylococcus was coagulase-negative.

Bacteria of the genus *Enterococcus spp*. were isolated 43 times from 15.03% of the birds. *Streptococcus spp*. was isolated 40 times from 13.99% of the birds.

Determination of pathogenicity was conducted for the following cultures of microorganisms: *Acinetobacter spp.* – 14 isolates, *Alcaligenes spp.* – 6 isolates, *Bordetella spp.* – 1 isolate, *Diplococcus spp.* – 2 isolates, *Flavobacterium spp.* – 2 isolates, *Corynebacterium spp.* – 7 isolates, *Hafnia alvei* – 1 isolate, *Klebsiella spp.* – 6 isolates, *Nocardia asteroides* – 5 isolates, *Pasteurella spp.* – 2 isolates, *Providencia spp.* – 1 isolate, *Pseudomonas spp.* – 5 isolates, *Sarcina spp.* – 4 isolates, *Stenotrophomonas maltophilia* – 1 isolate. From all investigated cultures 5 out of 6 isolates of *Alcaligenes spp.*, 1 isolate of *Bordetella spp.*, 2 out of 7 *Corynebacterium spp.*, 5 out of 6 *Klebsiella spp.*, 2 out of 2 *Pasteurella spp.*, 5 out of 5 *Pseudomonas spp.* and 1 isolate of *Stenotrophomonas maltophilia* were pathogenic. The rest isolates, which were put in control in the bioassay, were not pathogenic.

The structural composition of selected fungal flora in most cases represented by cultures of genera *Candida spp., Penicillium spp.* and *Aspergillus spp.*

In 28 specimens of 286 budgerigars fungal microflora was found in bacteriological material taken from the cloaca. The genus *Candida spp*. was the main representative of fungi. In material from pharynx, indication of fungal flora was in 59 cases. The largest number of the isolates of fungal microflora belonged to the genus *Candida spp*. - 29 cases. It should be noted that this representative is able to cause the severe forms of the candidiasis of birds. *Aspergillus spp*. represents the greatest threat to birds among the isolated fungal microflora and can cause aspergillosis. Other representatives of the fungal microflora are not usually causative agents of serious fungal infections.

Thus, the obtained data allow to note that fungal flora in the pharynx is represented in more cases than in the intestine. The detection rate of pathogenic fungal flora (*Candida spp.* and *Aspergillus spp*.) from the pharynx of budgerigars is 12.9% and 6.6% from the cloaca.

The isolates of bacteria genera *Diplococcus spp.*, *Capnocytophaga spp.*, *Citrobacter spp.*, *Moraxella osloensis*, *Nocardia asteroides*, *Providencia spp.*, *Sarcina spp.*, *Serratia spp.*, *Yersinia enterocolitica* did not have pathogenic properties that possibly indicating that they are saprophytic and symbiont representatives of the microflora of the digestive tract of budgerigars [13]. From an indicated fungal microflora *Candida spp.* and *Aspergillus spp*. represent particular danger to birds, which mostly isolated from the pharynx of birds.

Determining the status of saprophytic microflora of the digestive tract of birds has important diagnostic value. In addition, detection of possible bacterial carriage among exotic and decorative birds, being in close contact with the people, is essential to prevent the emergence and spread of infection.

CONCLUSION

The studies found that the normal bacterial microflora of the gastrointestinal canal of budgerigars is represented by bacteria of the genera: *Lactobacillus spp.*, *Staphylococcus spp.* (except coagulase-positive species), *Streptococcus spp.* (except hemolytic species), *Enterococcus spp.*, *Micrococcus spp.*, *Bacillus spp.*, *Enterobacter spp.*, *Escherichia coli*. To the saprophytic opportunistic causative agents should be attributed the bacteria of the genera *Acinetobacter spp.*, *Alcaligenes spp.*, *Haemophilus spp.*, *Corynebacterium spp.*, *Klebsiella spp.*, *Morganella osloensis*, *Pasteurella spp.*, *Pseudomonas spp.*, *Stenotrophomonas maltophilia*. Interest in the last is that these microorganisms did not cause clinical signs of disease in the birds, which in turn may indicate the possible bacterial carriage of parrots.

REFERENCES

1. Lenev S.V. et al. Improvement of allocation and identification of salmonella enterica bacteria of arizonae subspecies // Russian Journal of Agricultural and Socio-Economic Sciences, 2016; 2(50):14-23.
2. Bessarabov B.F. Practicum on diseases of birds // For students enrolled in the specialty "Veterinary Medicine" / Moscow, 2005. Textbooks and teaching aids for students in higher education.
3. Elisângela de Souza Lopes at all. Prevalence and Antimicrobial Resistance Profile of Enterobacteria Isolated from Psittaciformes of Illegal Wildlife Trade. Acta Scientiae Veterinariae, 2015; 43:1313.
4. Vasilevich F.I. Questions of veterinary and veterinary biology // Moscow State Academy of Veterinary Medicine and Biotechnology – MVA by K.I. Skryabin. Moscow, 2013.
5. Pimenov N.V. Perfection of means and methods of combating salmonellosis of birds // Veterinary and feeding, 2012. 4:32-34.
6. Harris, D.J., Oglesbee, B. L. Avian infection diseases. Manual Saunders: Clínica de pequenos animais. São Paulo: Roca, 2006. - p. 1740-1757.
7. Kathryn E. at all. Investigation and control of an attaching and effacing escherichia coli outbreak in a colony of captive budgerigars (Melopsittacus undulatus). Journal of Zoo and Wildlife Medicine, 2014; 4(45):875-882.
8. Flammer, K., Drewes, L.A. Species-related differences in the incidence of Gramnegative bacteria isolated from the cloaca of clinically normal psittacine birds. Avian Diseases, 1988. 1(32):79-83.
9. Medani, G. G. at all. Studies on some bacterial isolates affecting budgerigars. SCVMJ, 2008; XIII (1):37-48.
10. Bangert R.L., Cho, B.R., Widders, P.R., Stauber, E.H., Ward, A.C.S. A survey of aerobic bacteria and fungi in the feces of healthy psittacine birds, Avian Diseases, 1988; 1(32):46-52.
11. David N. at all. Implications of Macrorhabdus in Clinical Disorders. Charter 30. P. 706-710.

12. Lian Chai Bong. The bacterial flora of the upper respiratory tract in budgerigars and peaceful doves in captivity. Universiti Putra Malaysia. 1997.
13. Pimenov N.V. Prospects for the use of bacteriophages in veterinary // Veterinary and feeding, 2009; 5:34-36.
14. Collar N.J. at all. Handbook of the Birds of the World, v.4, Barcelona: Lynx Edicions, 1997, p. 280- 477.
15. Earle K.E. at all. The Nutrition of the Budgerigar (Melopsittacus undulatus). The Journal of nutrition, 1991; 121:186-192.
16. Laishevtcev A.I. Microbial profile of the digestive canal of budgerigars (Melopsittacus Undulatus) // Russian Journal of Agricultural and Socio-Economic Sciences, 2016; 5(53):76-82.
17. Nikolai Pimenov, Yulia Kolesnikova, Aleksey Laishevchev, Mohammad Ali Shariati, Aleksey Glinushkin, Aleksey Goncharov. Etiology and clinico-morphological manifestation of anaerobic enterotoxaemia of young cattle. Int. J. Res. Ayurveda Pharm. Mar - Apr 2016;7(Suppl 2):228-231 http://dx.doi.org/10.7897/2277-4343.07293

Cite this article as:

Laishevtcev Aleksey, Pimenov Nikolai, Tuhfatova Regin. The results of the identification of bacteria isolated from the digestive tract of birds *Melopsittacus undulatus* of home content. Int. J. Res. Ayurveda Pharm. Jul - Aug 2016;7(Suppl 3):147-151 http://dx.doi.org/10.7897/2277-4343.074173

Source of support: Nil, Conflict of interest: None Declared