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BIOLOGICAL PROPERTIES OF *CAMPYLOBACTER* MUSEUM STRAINS AFTER LONG STORAGE IN LYOPHILIZED FORM

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Summary. Campylobacteriosis is dangerous disease of agricultural animals, which is characterized by lesions of the reproductive system and the gastrointestinal tract. Contaminated livestock products can be a source of infection for humans and cause toxicoinfection with severe disease. Domestic preparations for the in vivo diagnosis of the disease is missing.

The main condition for the creation of effective diagnostics and vaccines is the presence of stable production strains. An important focus in the work of veterinary microbiologists is the studying biological properties of *Campylobacter* museum strains and definition of the capacity for preservation of these properties during long storage.

We conducted experiments for determine of museum *Campylobacter* cultures viability, that have been isolated from animals and birds and were stored in lyophilized form for different terms. A total of 24 strains were studied.

It has been established that *Campylobacter* lose about 1.0% of their life potential for every year of storage in lyophilized form. After 10–12 years of storage only 44.4% strains retain their viability, therefore it is not feasibly to keep them for a longer time under such conditions.

It has been shown the ability to save typical properties of Campylobacter strains within the specified retention period.

Keywords: *Campylobacter* strains, biological properties, viability

Introduction. Campylobacteriosis is zoonotic infectious disease of animals and people, characterized by various manifestations (genital impression, temporary infertility, abortion, diarrhea). This disease is caused by bacteria of *Campylobacter* genus (Mshelia et al., 2010; Swai, Hulsebosch and Van der Heijden, 2005).

According to WHO, campylobacteriosis is widespread in the world and causes 15% of all acute intestinal infections of animals and people. The most significant natural reservoirs of pathogens are livestock and poultry. The disease results in high significant economic losses in livestock farms by losing of adult animals their reproductive qualities (van Bergen et al., 2005; Mai et al.; Molina et al., 2013).

There are no means for animal campylobacteriosis diagnosis and prevention in Ukraine. The main condition for the creation of effective diagnostics and vaccines is the presence of stable production strains. Therefore, an important focus in the work of veterinary microbiologists is studying the biological properties of *Campylobacter* museum strains and definition of the capacity for preservation of these properties during long storage (Babkin, Galishchev and Novakovskiy, 2002, 2003).

Materials and methods. *Campylobacter* museum strains were stored in lyophilized form in vials under vacuum at minus 2–8 °C. The contents of the vials were plated on nutrient media — MPB, semisolid MPA with antibiotics (cephalothin, fuzydin), Campilobacahar, blood MPA. Strains were cultured under microaerophilic conditions (5% oxygen, 10% CO_2, 85% nitrogen) at 42 and 37 °C, within 72 hours. Accounting growth was performed visually every day, after 72 hours — by smear microscopy.

When typical growth was found (small transparent colonies with a grayish tinge to MPA, and as gray-blue rings on the surface of the semisolid MPA) there done smear (Gram and Stamp staining). At revealing the typical morphology (Gram positive spiral S-shaped sticks) further studies were carried out on the biochemical properties (formation of H_2S and indole, growth in semisolid MPA with 1% glycine, growth in semisolid MPA with 1% bile, growth in semisolid MPA with 0.02% cysteine, growth in semisolid MPA with 3.5 % NaCl, catalase production, sensitivity to nalidixic acid and cephalothin, hydrolysis of sodium hippurate, growth in semisolid MPA with 15, 25, 37 and 42 °C).

Results. We conducted viability determination of museum *Campylobacter* cultures, that have been isolated from animals and birds and were stored in lyophilized form for different periods. Totally, 24 strains were studied. We spent 25 consecutive passages on nutrient media and did not determined the presence and intensity of cultures growth in any case after opening the vials. The research results are presented in Table 1.

We were able to revive only 7 of the 24 strains. We found that, 3 of the 15 cultures only, were kept during 33–34 years, were viable for 25 passages.

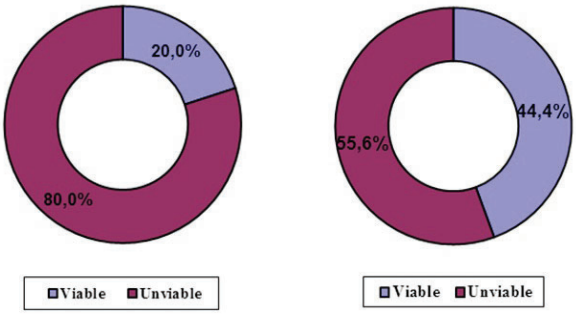
All of them belonged to the species *Campylobacter fetus* subsp. *venerealis*. 12 other cultures are kept their properties during 1–3 passages, but growth on nutrient media was absent.

Table 1 – Determination of the viability of *Campylobacter* cultures after long storage in lyophilized form

№	Name of strains	Term of the storage	Number of productive passages
1	*Campylobacter fetus* subsp. *venerialis* 4390	35	1
2	*Campylobacter fetus* subsp. *venerialis* 3121	33	3
3	*Campylobacter fetus* subsp. *venerealis* 1943 NaCl	35	1
4	*Campylobacter fetus* subsp. *venerealis* 60-312	34	1
5	*Campylobacter fetus* subsp.*venerealis* 2095	34	1
6	*Campylobacter fetus* subsp. *venerealis* 2095/2	33	2
7	*Campylobacter fetus* subsp. *venerealis* 2088	33	25
8	*Campylobacter fetus* subsp. *venerealis* 1707	33	1
9	*Campylobacter fetus* subsp. *venerealis* Rus	33	25
10	*Campylobacter fetus* subsp. *venerealis* 3816	34	1
11	*Campylobacter fetus* subsp. *fetus* 17	33	1
12	*Campylobacter fetus* subsp. *fetus* 372	11	3
13	*Campylobacter fetus* subsp. *fetus* 9SV	12	25
14	*Campylobacter fetus* subsp. *fetus* 2BGV	11	3
15	*Campylobacter fetus* subsp. *venerialis* 6913	33	25
16	*Campylobacter jejuni* 5779	10	25
17	*Campylobacter fetus* subsp. *venerialis* 6913/2	11	25
18	*Campylobacter fetus* subsp. *fetus* 15OV	10	25
19	*Campylobacter fetus* subsp. *fetus* 5OV	11	10
20	*Campylobacter jejuni* 5779	33	2
21	*Campylobacter jejuni* 5779/2	12	3
22	*Campylobacter jejuni* 5779/3	11	2
23	*Campylobacter fetus* subsp. *intestinalis* 240	33	1
24	*Campylobacter fetus* subsp. *intestinalis* 1767	34	1

Of 9 cultures, that were stored during 10–12 years, the were viable only 4. They belonged to the 3 species — *Campylobacter fetus* subsp. *fetus*, *Campylobacter fetus* subsp. *venerealis* and *Campylobacter jejuni*. None of the cultures of *Campylobacter fetus* subsp. *intestinalis* restore failed.

Thus, we have been a direct correlation between the degree of viability of the strains of their age (Figure 1).



33–35 years of storage 10–12 years of storage

Figure 1. Viability of *Campylobacter* strains

After 33–35 years storage, 20.0% of the strains only were viable. Whereas among the strains, that were stored during 10–12 years, this index was almost twice as much and was 44.4%. We calculated that the culture lose about 1.0% of their life potential for every year of storage.

For future work, we have selected three strains that were stored during 10–12 years. We examined their biochemical and cultural properties in a series of experiments (Table 2).

Table 2 – Biochemical and cultural properties of *Campylobacter* strains

№	Test	*C. fetus* subsp. *fetus* 15OV	*C. fetus* subsp. *venerialis* 6913/2	*C. jejuni* 5779
1.	Catalase production	+	+	+
2.	Growth with temperature 15 °C 25 °C 37 °C 42 °C	 – + + +/–	 – + + –	 – – – +
3.	Growth in semisolid MPA with 1% glycine	–	+	+
4.	Growth in semisolid MPA with 1% bile	+	+	+
5.	Growth in semisolid MPA with 3.2% cysteine	+	+	+
6.	Growth in semisolid MPA with 1.5% NaCl	–	–	–
7.	Sensitivity to nalidixic acid Sensitivity to cephalothin	– +	– +	+ –
8.	Formation of H_2S	+	+	+
9.	Formation of indole	–	–	–
10.	Hydrolysis of sodium hippurate	–	–	+

«+» — the presence of growth;
«–» — no growth; «+/–» — insignificant growth

We found that *Campylobacter* strains have retained typical properties. All 3 strains produced catalase; grew up with temperature 15 °C; grew up on in semisolid MPA with 1% bile and 3.2% cysteine; did not grow in semisolid MPA with 1.5% NaCl; form H_2S; did not form of indole.

C. fetus subsp. *fetus* 15OV and *C. fetus* subsp. *venerialis* 6913/2 grew up at 25 and 37 °C; were not sensitive to nalidixic acid and were sensitive to cephalothin; hydrolysed of sodium hippurate. *C. jejuni* 5779 did not grew up at 25 and 37 °C; grew up on in semisolid MPA with 1% glycine; were sensitive to nalidixic acid and was not sensitive to cephalothin; not hydrolysed sodium hippurate.

All strains had various sensitivity to temperature of 42 °C — *C. fetus* subsp. *fetus* 15OV showed insignificant growth, *C. fetus* subsp. *venerialis* 6913/2 did not grow and *C. jejuni* 5779 grew up at this temperature.

Conclusions. It was established that *Campylobacter* lose about 1.0% of their life potential for every year of storage in lyophilized form. After 10–12 years of storage only 44.4% strains retain their viability, therefore it is necessary to consider these storage conditions.

It has been shown the ability to save typical properties of *Campylobacter* strains within the specified retention period.

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