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Occurrence of Campylobacters in the food-chain and effect of environmental factors on their survival and destruction

Brief version of the PhD thesis

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Introduction, objectives

One of the most important risk factors of food related human diseases is the microbial contamination. In past decades the food industry made efforts to eliminate most of the classic microbial causative agents, but parallel to this they had to face a new risk: bacteria causing mild to moderate symptoms to humans, but with higher incidence and economic loss like *Salmonella* and *Campylobacter* species. From this group of bacteria *Salmonella* species have been the leading human enteric illness causatives for several years, but in the recent past more and more *Campylobacter* caused human diarrhoeal diseases were observed.

This trend is observable in global as well, so not surprisingly in 2000 the WHO designated the *Campylobacter* species (especially *Campylobacter jejuni*) as the most important source of enteric zoonoses in developed countries.

While in developing countries the not properly treated water and the contact with animals are the leading risk factors, in developed countries, so in Hungary the sources of infections show a more complex view. In the developed countries the prioritized risk factors below show the most important possibilities of infection:

1. handling and consuming of poultry meat
2. food of animal origin, raw milk
3. not properly treated drinking water
4. contact with pet and farm animals
5. travels abroad (travel diarrhoea).

One of the reasons of infections of poultry origin is the improper heat treatment of meat and the increasing consumer demand for raw foods. During the slaughter technology of poultry in the phases of plucking and evisceration the surface of meat can be significantly contaminated. The most important hazards, which are the very common during poultry processing, are the not adjusted eviscerating equipment and the excessive processing speed.

Despite that in Hungary an increasing attention is paid to the prevention of microbial foodborne diseases, the number of foodborne gastroenteric cases is not decreasing significantly. In Hungary the leading sources of foodborne enteric zoonotic diseases are *Campylobacter* and *Salmonella* species, the yearly reported cases of human

campylobacteriosis exceeded salmonellosis. The vast majority of cases are sporadic ones, mass infections related to *C. jejuni* are reported rarely.

Campylobacter jejuni is a normal member of gut flora and often can be found in intestines of domestic and wild animals, in the mouth cavity of animals and people. During meat processing due to fecal contamination the bacteria can appear in food of animal origin. Sporadically they can cause abortion in ruminants, more rarely diarrhoea in newborn calves, and mastitis in dairy cows. During mastitis the bacteria are permanently transferred to milk causing *Campylobacter*-enteritis in people after consumption.
In the intestines of poultry and in wild birds *C. jejuni* is ubiquitous, and except for the domestic hen in which it causes *Campylobacter*-hepatitis, there are no symptoms. So the screening of carrier animals at slaughter is nearly impossible. *Campylobacter jejuni* cannot pass from the intestines to the eggs, so the chicks are free from infection.

Due to literature the optimal temperature for growth of *Campylobacters* is at 42℃, but the microbe is able to multiply between 30.5−45.0℃.

Campylobacters grow relatively slowly, the pH optimum is between 6.5–7.5, but is able to survive between 4.9−9.0. It is a microaerophilic organism, which means it requires 3 to 15% oxygen and 3 to 5% carbon dioxide for optimal growth conditions. The optimal water activity is $a_w = 0.997$ (set with 0.5 % NaCl), and the minimum is $a_w = 0.987$ (set with 2.0 % NaCl).

Despite that *Campylobacter* species are described as sensitive to small changes of environmental factors in the suboptimal direction, they seem to be able to survive permanently in food and to remain infectivity. To the appropriate control we need to know the spreading mechanism of *Campylobacter jejuni*, and for this there is a need for from-farm-to-fork investigations and experiments concerning the characteristics of the microbe. We do not know much about the way the *Campylobacter* infects the broiler flocks, the reaction of *Campylobacter jejuni* to the environmental factors and the possibilities of elimination of the bacteria from the food chain. Due to these facts it became obvious, that the most important role in defeating campylobacteriosis has the prevention of infection. With appropriate application of HACCP system and keeping the criteria of Good Hygienic Practice (GHP) the risk of contamination can be decreases largely.

For the prevention of diseases caused by *Campylobacter jejuni* food preservation procedures should be developed, which prevent bacterial growth, possibly may destroy the microbes, and does not adversely affect the organoleptic properties of food. The changes of

environmental conditions (temperature, water activity, and pH) can be used to decrease the growth of bacteria, so these methods may be the most important tools for combating foodborne human campylobacteriosis. To answer these important questions farm-to-fork investigations and experiments on the characteristics and the properties of the microbe are needed.

In the thesis an investigation of the occurrence of *Campylobacters* in two Hungarian broiler flocks from first day of life to the slaughter of the animals is presented, looking for answers regarding transmission properties within flock and seasonality.
Then experiments are presented on how the environmental factors, like temperature, water activity and pH, as well as combinations of these changes, impacts the bacterial growth, survival and death. During the investigations suitability for evaluation of survival and death trials of a new rapid microbiological living cell determination method, based on redox-potential measurement, is examined.

Materials and methods

The experiments were carried out in the accredited Microbiological Laboratory of the Food-hygiene Department of Szent István University Faculty of Veterinary Science. We used *Campylobacter jejuni* strains isolated from poultry slaughterhouse for the experiments. The microbe was maintained in selective Bolton broth (MERCK 1.00068) with weekly inoculation.

Experimental samplings on farm and slaughterhouse spreading mechanisms were carried out in a broiler flock and a slaughterhouse of Hajdú-Bét Co., Hungary. Two sampling periods were planned – first in summer, second in winter – to obtain information about any seasonal differences in the way or the rate of infection. According to the sampling design samples were taken when the animals were transported to the farm from the hatchery, and then every two weeks till slaughtering, from the same flock. At the slaughterhouse, samples were taken from different points of the slaughter line.

Samples at farm and slaughterhouse were taken with a sterile swab from the cloaca, surface of the wall near the ventilation aperture (10 cm^2), personnel's hand, surface of the carcass and the environment. The swabs were immediately put into 10 ml transport selective enrichment broth (Preston broth: Nutrient Broth No.2 – Oxoid CM0067; Campylobacter Growth Supplement – Oxoid SR0084; Preston Campylobacter Selective Supplement – Oxoid SR117E). Insects, samples from the animal feeds and from the deep litter were taken to sterile Stomacher-bags, and were transported to the laboratory where they were mixed with enrichment broth. Air samples were taken with Koch sedimentation method with 15 minutes exposition time to selective mCCDA agar (Campylobacter Blood Free Selective Agar Base – Oxoid CM0739 and CCDA Selective Supplement – Oxoid SR0155). Water samples were taken from the water tap – 1 minute after turning it on – to sterile bottles.
The sample analysis was carried out according to Hungarian standard MSZ-3640/24-1989. The samples in the selective enrichment broth (Preston broth) were incubated for 48 hours at 42℃, and then they were spread on the surface of s elective modified charcoal cefoperazone deoxycholate agar (mCCDA). The plates were incubated at 42℃ under microaerophil conditions (Anaerocult®C – MERCK 1.16275). Water samples were filtered through 0.30µm pore size membrane, and then put on mCCDA agar plates. The strains were identified on the basis of the standard.

During experiments on effect of environmental factors (temperature, pH, and water activity) two living cell determination methods were used: classical culture method and a rapid method based on measurement of redox-potential.
The classical culture method for determination of living cell number of *Campylobacter jejuni* is based on standard ISO/TS 10272-2:2006. Decimal dilution series was made from the sample in salt-peptone water (8.5 g/l NaCl, 1 g/l peptone). 0.1 ml from every member of the dilution series was spread on mCCDA agar (MERCK 1.00070). The plates were incubated at 42℃ for 48 hours in anaerobe jar (MERCK 1.16387) u nder microaerophil conditions set with MERCK Anaerocult C (MERCK 1.16275).

The living cell number determination of *Campylobacter jejuni* based on redox-potential measurement was performed with a method patented by experts of Szent István University Faculty of Veterinary Science Department of Food Hygiene and Corvinus University of Budapest Faculty of Food Science Department of Physics and Control and equipment called MicroTester working according to these patented principles.

The equipment detects the growth of microbes based on measurement of redox-potential of culture medium. The energy source of the microbial growth is the biological oxidation which results in a reduction in the environment. This is due to the oxygen depletion and the production of reducing compounds in the nutrient medium. The redox potential is one of the most complex indicators of the physiological state of microbial cultures and its measurement could be a useful tool for the qualitative and quantitative determination of the microbial contamination.

There is a strong linear correlation between the logarithm of different original living cell concentrations (lgN) and the Times To Detection (TTD) belonging to them, it makes possible to obtain a calibration curve.
From the *Campylobacter jejuni* strain to be tested decimal dilution series is made with peptone water. The original living cell concentration is determined by classical culture method describe previously. From every member of the dilution series 1 ml is added to 100 ml sterile Bolton broth (Merck 1.00068 and Bolton selective supplement – Merck – 1.00069) in the MicroTester measurement cells, then incubated at 42℃ the TTD is determined. From the lgN obtained by classical culture method and from the TTD values obtained by redox-potential measurement the equation of the calibration curve is calculated by linear regression, which is entered to the equipment software. In the possession of the calibration equation later we will have a possibility to determine the original living cell number of a specified sample.

For determination of the original living cell concentration of *Campylobacter jejuni* – after conventional microbiological sample preparation (homogenizing, diluting) – a known amount is placed in the test cell and the measurement is carried out. As a negative control sterile medium, as a positive control a test cell inoculated with a known concentration of the tested microbe is used. The redox curve is registered, the TTD is determined and with the calibration equation the original cell concentration is calculated by the equipment.

During classical isotherm heat destruction experiments carried out at different temperatures the death parameters calculated from original viable cell number obtained by classical culture method and by the redox-potential method were compared. During the isotherm heat destruction experiments carried out at different temperatures the test tubes containing known cell number (N_0) suspension (9 ml 0.5% glucose solution inoculated with 1 ml *Campylobacter* suspension) were placed in water bath of Medingen U10 ultrathermostate. In the previously determined sampling times 3-3 parallel suspension samples were taken, then the probes were put into cold water, and then 1 ml was measured into Bolton broth. The broth was incubated at 42℃ for 48 hours, then it was plated out onto a mCCDA agar surface; and it was incubated at 42℃ for 48 hours at microaerophil circumstances.

In case of classical culture method heat destruction time (τ) is defined as the shortest heat treatment time when there are no surviving cells from 3 parallel samples. From the heat destruction times the decimal reduction times can be calculated. In case of the redox-potential method the surviving cell numbers (lgN_t) belonging to different heat treatment times were determined by a 16-channel MicroTester equipment with the previously determined calibration curve. The decimal reduction times (D_T) belonging to different temperatures were calculated from the initial viable cell concentration (N_0) and from the cell number surviving (N_t) a *t* time heat treatment.

The effect of water activity on survival of *Campylobacter jejuni* was examined by determination of surviving cell numbers with redox-potential method. The water activity was set with three different materials (NaCl, glucose and glycerine), the experiments were carried out in the water activity range typical for food. 10 ml of *Campylobacter jejuni* suspension enriched for 24 hours in Bolton broth was measured to previously prepared sterile NaCl-, glucose- and glycerine-solutions thermostated at 42℃. After mixing 1 ml samples were taken at 0, 15, 30 and 45 minutes, and were put into 42℃ Bolton broth redox measurement cells. The redox equipment determined the TTD values and calculated the original living cell concentration of *Campylobacter jejuni* based on calibration curve.

In further experiments the effect of pH on survival of *Campylobacter jejuni* was investigated, the survived cell fraction was determined by MicroTester equipment. The experiments were carried out in pH range 4.5 – 10. 1 ml of *Campylobacter jejuni* suspension enriched for 24 hours in Bolton broth was measured to previously adjusted (pH, water activity) Bolton broths. For adjustment of water activity glycerine was used, in addition at optimal pH 7.2 NaCl was used as well. The pH was adjusted with NaOH and sterile tartaric acid. The prepared test cells were immediately connected to the redox equipment.

Results

During farm and slaughterhouse samples to obtain an overview of spreading of *Campylobacter* species within a flock samples were taken from broiler flocks from settling till slaughter in the summer and in the winter as well (total 195 samples). In the summer on the first two sampling days (day 0 and day 12) all samples (70) were negative. On day 26 one cloaca sample, one sample from the surface of the wall near the ventilation aperture and an insect-sample was positive. On day 42 *Campylobacter*s could be detected on every sampling point (90 samples) at the slaughterhouse.

In the winter sampling period, none of the farm samples were positive. On day 42, we found *Campylobacter*s on every sampling point at the slaughterhouse. A total of 93.3% of the live animals' cloaca and 100% of the carcass surface samples after plucking, after washing and after chilling were positive. We could isolate *Campylobacter jejuni* from the surface of the staff's hands and from the samples from the slaughtering equipment as well. Out of the *Campylobacter* positive samples 95.5% (84 of 88) were infected with *Campylobacter jejuni*.

During examination of thermal death of *Campylobacter jejuni* we started the tests from the same basic suspension with original living cell concentration $N_0=1{,}0\cdot10^5$ cfu/ml. The heat destruction times and decimal reduction times calculated belonging to different temperatures are presented in *Table 1.*

Table 1. Heat destruction characteristics of *Campylobacter jejuni* obtained by culture method

Temperature **T (℃)**	**Original cell N** **lg N_0**	**Heat destruction time** **τ (min)**	**Decimal reduction time** **D (min)**	**lg D**
50	5.0	60	12.0	1.079
55	5.0	20	4.0	0.602
60	5.0	8	1.6	0.204
65	5.0	3	0.6	-0.222

For the redox-potential based experiments first the calibration curve was determined. The redox-curves determined from the decimal dilution of the initial suspension are presented on *Figure 1.*

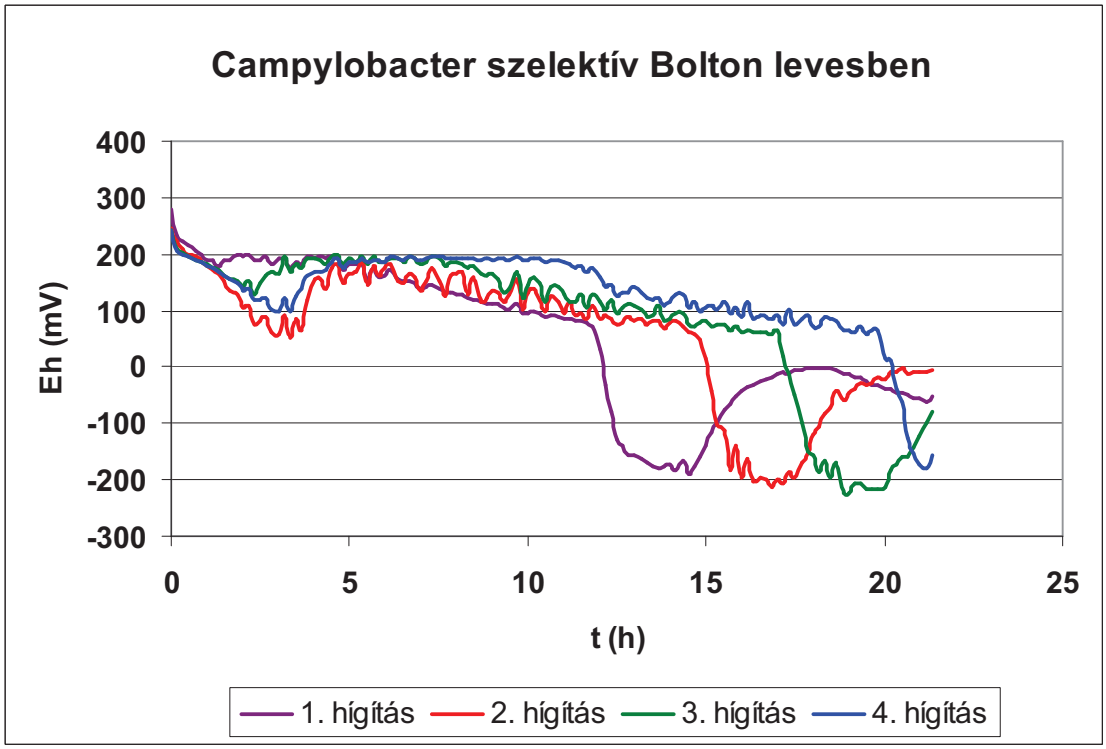

Figure 1. The effect of dilution on redox-curves of *Campylobacter jejuni*

The TTD values belonging (detectation criterion -0.5 mV/min) to different dilutions compared to the cell numbers obtained by spreading method on mCCDA agar the calibration curve and the calibration equation can be determined. The results of two independent measurement series are presented in *Table 2*., the joint calibration curve determined upon these data is presented on *Figure 2*.

Table 2. TTD values of *Campylobacter jejuni* as a function of original cell concentration

lgN	TTD (h)
7.6	12.00
6.6	14.83
5.6	17.17
4.6	20.00
6.0	17.17
5.0	19.17
4.0	22.00
3.0	24.83
2.0	28.67

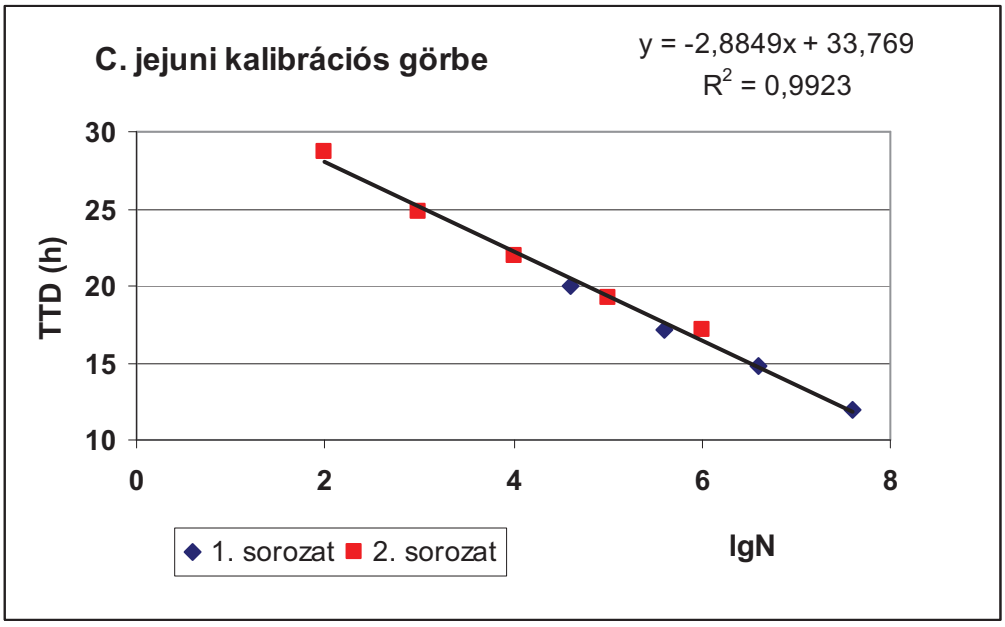

Figure 2. Calibration curve of *Campylobacter jejuni*

The decimal reduction times calculated from survived cell numbers determined with redox-potential measurements are presented in *Table 3*.

Table 3. Heat destruction characteristics of *Campylobacter jejuni* obtained by redox method

T (℃)	lg N_0	Heat treatment (min)	lg N_t	D (min)	lg D
52	7.25	10	5.89	7.35	0.866
56	7.25	5	5.36	2.65	0.423
60	7.25	4	4.36	1.38	0.141
64	7.25	2	4.23	0.66	-0.179

During further experiments we examined the effect of environmental factors – like water activity and pH – on the growth and survival of the microbe. In the tests carried out we examined the survival of *C. jejuni* at optimal 42℃ at six different water activity le vels (0.995, 0.985, 0.976, 0.946, 0.920, and 0.874). The water activity was set with three substances (NaCl, glucose, glycerine), so we could observe the effect of different materials on *C. jejuni* at same water activity levels. At water activities 0.995, 0.985 and 0.976 the cell number did not change after 45 minutes in every substance solution. At water activity 0.946 there were differences between the solutions. In NaCl solution a large and fast microbial death was observable, there were no surviving cells in 0. minute sample. At water activity 0.920 we examined cell count in glycerine and glucose solutions. At water activity 0.874 we observed immediate and total bacterial death, so no more examinations were made at this level.

During the examination of the effect of pH we tested the growth of *Campylobacter jejuni* in selective Bolton broth in pH range 4.5 – 10, with 0.5 pH steps. The measurements were carried out at 42℃ with redox-potential measuremen t technique. The pH was adjusted with NaOH and sterile tartaric acid. There was 80 ml broth in the test cells which were inoculated with 1 ml 48 hours *Campylobacter* suspension. The original living cell concentration of the suspension was $2.3 \cdot 10^6$ cfu/ml. The measurement was carried out for 48 hours. The results are shown in *Table 4.*

Table 4. Effect of pH on growth and survival of *Campylobacter jejuni* (T=42 ℃)

pH	TTD (h)	survival
4.5	-	-
5.0	-	+
5.5	25.33	+
6.0	19.17	+
6.5	12.83	+
7.2	13.5	+
7.5	14.33	+
8.0	17.33	+
8.5	-	+
9.0	-	-
9.5	-	-
10.0	-	-

From the table we can observe that *Campylobacter jejuni* is able to grow in pH range 5.5 – 8.0. From test cells not showing growth we spread on mCCDA agar to determine if there were survived cells. In the table the samples showing growth on mCCDA agar are marked with +. The TTD values obtained in pH range 5.5 – 8.0 are shown in *Figure 3*.

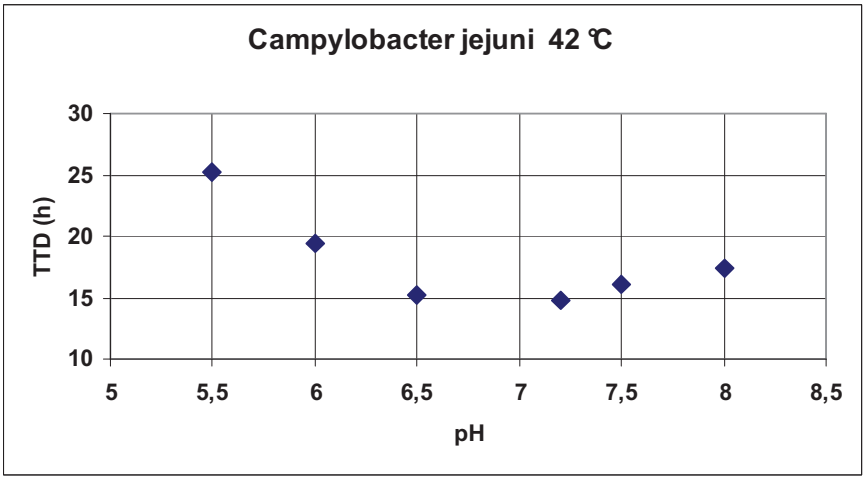

Figure 3. Changes of TTD values of *Campylobacter jejuni* as a function of pH (T=42 ℃)

The combined effect of pH and temperature was examined in selective Bolton broth in pH range 5.5 – 8.0, with pH 0.5 steps at 27, 32, 37, 42, and 48 ℃ with redox-potential measurement. The results are presented in *Table 5.*

Table 5. The effect of pH and temperature on growth and survival of *Campylobacter jejuni*

T (℃)	pH	TTD (h)	survival	T (℃)	pH	TTD (h)	survival
27	5,5	-	+	42	5,5	25,17	+
	6	-	+		6	19,67	+
	6,5	-	+		6,5	17,67	+
	7,2	-	+		7,2	16,17	+
	7,5	-	+		7,5	17,83	+
	8	-	+		8	17,5	+
32	5,5	-	+	48	5,5	-	-
	6	-	+		6	-	-
	6,5	-	+		6,5	-	-
	7,2	-	+		7,2	-	-
	7,5	-	+		7,5	-	-
	8	-	+		8	-	-
37	5,5	25,17	+				
	6	19,33	+				
	6,5	18,83	+				
	7,2	20	+				
	7,5	20	+				
	8	18,83	+				

From the results it is observable that *Campylobacter jejuni* was able to multiply only at temperatures 37 and 42℃. From test cells not showi ng growth we spread on mCCDA agar to determine if there were survived cells. In the table the samples showing growth on mCCDA agar are marked with +. The TTD values measured at 37℃ are shown on *Figure 4.*

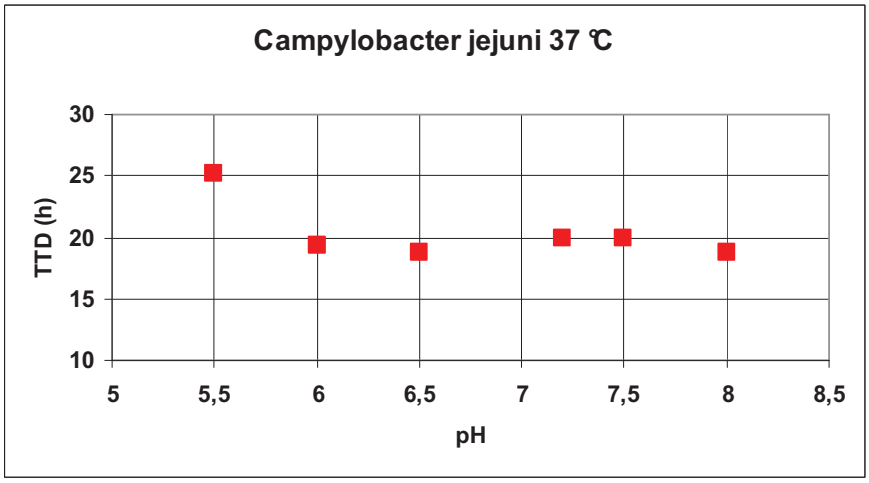

Figure 4. Changes of TTD values of *Campylobacter jejuni* as a function of pH (T=37 ℃)

The effect of water activity on growth and survival was measured in selective Bolton broth at water activities aw=0.985, aw=0.975, aw=0.965. The effect of water activity on growth was examined by setting water activity with glycerine and NaCl as well. The measurement was carried out at 42℃ with redox-potential measuremen t. Due to examination results there was no growth in these circumstances. From all test cells not showing growth we spread on mCCDA agar to determine if there were survived cells. We observed survival in all probes but in 0.965 NaCl adjusted test cell.

Because the microbe was not growing under decreased water activity and optimal pH and temperature environment, we could not examine the combined effect of water activity, pH and temperature.

Discussion

From the results of the surveys and the experiments investigating the microbial resistance to environmental factors discussed in the thesis the following conclusions can be drawn.

The results obtained during examination of occurrence of *Campylobacter jejuni* on farm and slaughterhouse are in a good agreement with international results: we could not detect *Campylobacter*s till day 26, but at day 42 (at slaughterhouse) the 93.3% of the live animals was infected, as all of the technological and personal hygiene samples as well. With the progress of the processing line we could isolate *Campylobacters* from all samples. Out of the positive samples 95.5% were detected as *Campylobacter jejuni*. These results confirm data which show that after infection of a flock almost every animal becomes carrier of the microbe, and which accidentally not, it will at slaughterhouse. The results showed that *Campylobacter* species were present in almost the whole flock, and were spread on the slaughter line. In this process the hygiene deficiencies of the equipment and the personnel could play role.

The results show how important is to prevent the poultry meat from infection with *Campylobacters* and the possible role of eradication programmes. The results also show an increased importance of further investigations on the epidemiology of the infection, mainly focusing on the possible routes of infection and the possibilities of prevention. Although there have been numerous studies investigating the occurrence of *Campylobacter* species and the number of *Campylobacter*-caused human gastro-enteric cases worldwide, there are only a few Hungarian published data on the real infection rate of poultry flocks and the rate within a flock, the effect of the slaughter and processing technology on the contamination of carcasses. The results presented in the thesis show high infection rate within the flock (46.6-93.3%) in good agreement with previous Hungarian data.

Due to our findings around fourth week of age the birds become infected. When a flock was infected, nearly every animal became infected with *Campylobacter* species. In studies there was a difference between the summer and winter infection rates of the broiler flocks, this is in good correlation with international results. In our study in summer period 85.8% of the slaughterhouse samples were positive, in winter 97.7%. All of the chilled carcass samples were positive in summer and in winter, as well. In our work 95.5% of the positive samples were infected with *Campylobacter jejuni*, it correlates with international data.

The results presented in the thesis indicate that the *Campylobacter* infection rate of Hungarian broiler flocks is similar to those reported from the other countries. The results, however, also show an increased importance of further investigations on the epidemiology of the infection, mainly focusing on the possible routes of infection and the possibilities of prevention.

During the examination of the effect of temperature on survival of *Campylobacter jejuni* we observed that the growth temperature range of the microbe is very narrow. We could observe multiplication only at 37 and 42 ℃, and we could n ot at 32 and 48 ℃. The microbe survived 32℃ for 48 hours, but at 48℃ the suspension with initial cell concentration of 10^6/ ml died during the experiment time (48 hours).

During classical isotherm heat destruction experiments carried out at different temperatures the death parameters calculated from original viable cell number obtained by classical culture method and by the redox-potential method were compared.
The lgD values obtained from culture and redox potential methods illustrated together as a function of temperature we can obtain a combined thermal death curve (*Figure 5.*)

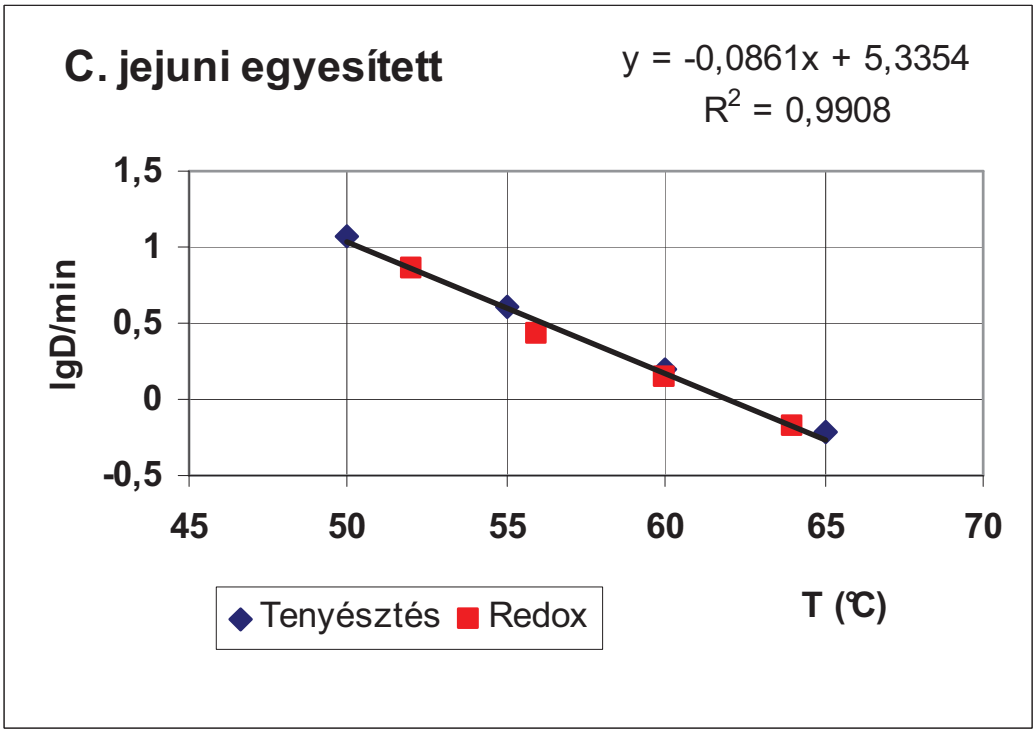

Figure 5. Combined thermal death curve of *Campylobacter jejuni*

The very tight fit thermal death curves prove that there is no significant difference between results obtained with classical culture method and redox-potential method.

The equation of the thermal death curve:	lgD = 5.34 – 0.0861·T
Standard error of lgD:	standard error = 0.0487
z value calculated from the slope:	z = 1/0.0861 = 11.6 ℃
The 95% confidence interval of the slope:	-0.0944 < -1/z < -0.0778
The 95% confidence interval of the z value:	10.6 ℃ < z < 12.8 ℃

Based on experiment results we can identify that the redox-potential rapid method is suitable for evaluation of heat destruction experiments. There is no significant difference between decimal reduction times obtained by these two different methods, so the results can be presented in a combined thermal death curve.

The redox-method is significantly faster, it lasts 25-30 hours comparing to 72 hours time demand of the culture method, with less work and culture media usage.

Based on thermal death curves we can state that the z value (temperature rise needed for the decrease of D value with one order of magnitude) of *Campylobacter jejuni* is higher than the usual 5 ℃ value for vegetative microbes. In ca se of *Campylobacter jejuni* the z value is 11.6 ℃, this high value is characteristic for spor e forming bacteria. However, the thermal tolerance (the decimal reduction time at a given temperature) not differs from the normal values of vegetative microbes.
Due to these results *Campylobacter jejuni* is not more heat tolerant than the vegetative bacteria in the examined temperature range 55-65 ℃ , but is less sensitive to the changes of temperature. This fact is important in the planning of heat treatment of food containing *Campylobacter jejuni.*

During experiments on effect of water activity on survival of *Campylobacter jejuni* the change of living cell number can be observed at different water activity levels set with different substances, letting us to draw conclusions on microbial death.
The changes of living cell concentrations calculated form lgN values determined with redox-potential measurement are presented in *Table 6*. lgN_0 means the logarithm of the living cell number of the sample inoculated, mixed and taken out immediately, ΔlgN is the difference between the initial and the final (after 45 minutes) values.

Table 6. Change of living cell concentration of *Campylobacter jejuni* as a function of water activity (45 minutes treatment at 42 ℃)

a_w	glycerine		glucose		NaCl	
	lgN_0	ΔlgN	lgN_0	ΔlgN	lgN_0	ΔlgN
0.995	7.5	0	7.5	0	7.5	0
0.985	7.5	0	7.5	0	7.5	0
0.976	7.5	0	7.5	0	7.5	-1.5
0.946	2.5	0	6	-3.8	0	-
0.920	1.5	-1.5	4.2	-3.4	-	-
0.874	0	-	-	-	-	-

At water activities 0.995, 0.985 and 0.976 the cell number did not change significantly after 45 minutes. So it can be stated that the microbe is able to survive a 0.976 water activity environment.

At water activities 0.946 and 0.920 the living cell concentration largely decreased in the moment of inoculation in case of all three substances. The level of decrease was the highest in case of NaCl, the cells practically died in the moment of inoculation.

In glycerine the initial fast decrease is followed by slower changes. This phenomenon could be explained with the fact that glycerine can go into the cytoplasm freely and it leads to quick osmotic balance.

The glucose had not significant immediate effect on the bacterial death, but the living cell concentration decreased constantly and at 45 minutes it was at the same level as in glycerine.

At water activity of 0.876 the microbe was destroyed within 15 minutes in case of glucose and glycerine as well, the microbe is sensitive for osmotic shock.

Summing up the *Campylobacter jejuni* is sensitive for changes in water activity, the microbe is not able to bear drying, salting and adding sugar. This fact is important in preservation processes of food containing *Campylobacter jejuni* based on decreasing water activity.

During the experiments on the effect of pH we found that *Campylobacter jejuni* is able to multiply in pH range of 5.5 – 8.0. The pH optimum of the microbe is around 7.2. At pH values above or below optimum the TTD needed for detection of initial cell concentration increases, so the growth speed decreases.

Although the microbe did not grow below pH=5.5 and above pH=8.0 but was able to survive circumstances between 5.0 – 8.5 and was able to grow after putting it in optimal conditions. Below pH=5.0 and above pH=8.5 the microbe was destroyed during the experiment (48 hours).

Campylobacter jejuni did not grow at water activities examined (between 0.985-0.965), but it was able to survive these conditions. The exception was the value of 0.965 set with NaCl in which case the microbe was destroyed during the experiment (48 hours).

Due to results that the microbe was not able to multiply at lower water activity values even in otherwise (pH, temperature) optimal conditions, we could investigate only the combined effect of temperature and pH and only at two temperatures (37 ℃ and 42 ℃).

We found that at suboptimal temperature near optimal pH values (6.5-7.5) the microbe is not sensitive to changes of pH. Going further from the optimum the sensitivity to the pH increases and the sensitivity to temperature decreases. The results are presented at *Figure 6.*

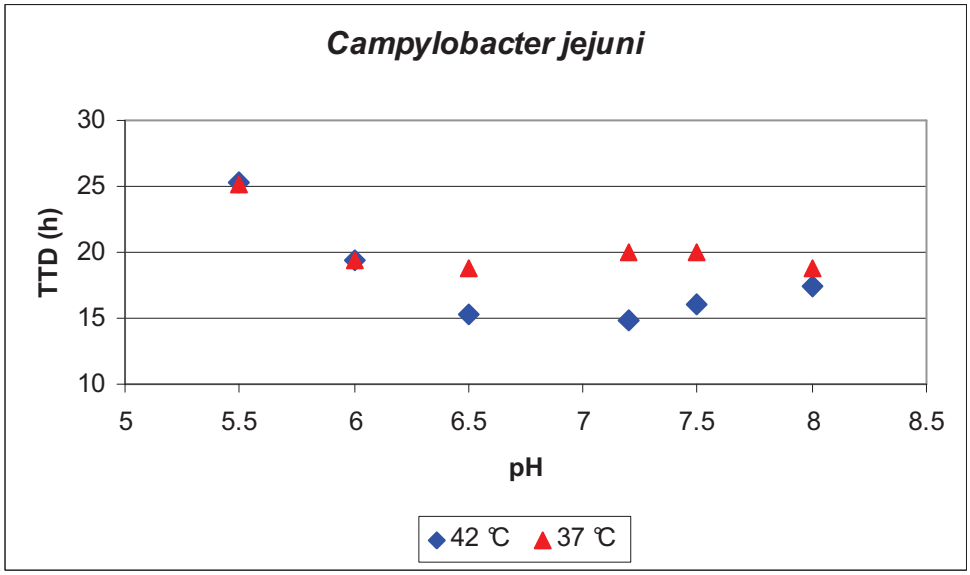

Figure 6. The changes of TTD values of *Campylobacter jejuni* as a function of pH and temperature

New scientific results

1. The results from farm and slaughterhouse samplings confirm data which show that after infection of a flock almost every animal becomes carrier of the microbe, and which accidentally not, it will at slaughterhouse. During farm samplings *Campylobacters* were isolated from the poultry farm environment as well.

2. In studies there was a difference between the summer and winter infection rates of the broiler flocks. Due to our findings around fourth week of age the birds become infected. The reason for this type of age dependence of infection is not really known yet, but may play an important role in prevention.

3. During examination of resistance of *Campylobacter jejuni* to environmental factors the applicability of a new, so far not used rapid microbiological method, the redox-potential measurement based living cell number determination, was investigated for death kinetics experiments. Due to results it can be stated that the new rapid method is applicable for evaluation of thermal death experiments. There was no significant difference between decimal reduction times obtained by the classical culture and the redox method, so the results could be presented on a joint thermal death curve.
   The redox-method is significantly faster, it lasts 25-30 hours comparing to the 72 hours time demand of the classical culture method, with less work and culture media usage.

4. Based on thermal death curves we can state that the z value (temperature rise needed for the decrease of D value with one order of magnitude) of *Campylobacter jejuni* is higher than the usual 5 ℃ value for vegetative microbes. In case of *Campylobacter jejuni* the z value is 11.6 ℃, this high value is characte ristic for spore forming bacteria. However, the thermal tolerance (the decimal reduction time at a given temperature) not differs from the normal values of vegetative microbes.
   Due to these results *Campylobacter jejuni* is not more heat tolerant than the vegetative bacteria in the examined temperature range 55-65 ℃, but is less sensitive to the changes of temperature. This fact is important in the planning of heat treatment of food containing *Campylobacter jejuni.*

5. During further experiments the effect of water activity on growth and survival of *Campylobacter jejuni* was investigated at different water activity levels set with different substances. With glycerine we could observe the effect of lower water activities. With glucose and NaCl we could model the circumstances in food preserved with sugar and salt, respectively.

   During the experiments we could determine that the microbe will not multiply below water activity of 0.995, but above 0.876 a_w value will not die and getting to optimal environment the bacteria could start to grow. In case when $a_w \leq 0.876$ the microbe will die.

   The survival chances of *Campylobacter jejuni* largely decrease using sugar or salt. In the former case at aw ≤ 0.946, in the latter at aw ≤ 0.965 the microbe will die within 48 hours.

6. During the experiments on the effect of pH we found that *Campylobacter jejuni* is able to multiply in a narrow pH range of 5.5 – 8.0, but can survive circumstances between 5.0 – 8.5 and is able to grow after putting the microbe in optimal environment. Below pH=4.5 and above pH=9.0 the microbe died within 48 hours.

List of publication related to the thesis

Jozwiak Á., Reichart O., Laczay P.: **The occurrence of Campylobacter species in Hungarian broiler chickens from farm to slaughter**. J. Vet. Med. B 53, 291–294., 2006. *(IF: 1,356)*

Jozwiak Á., Reichart O., Szakmár K.: **Redoxpotenciál-mérésen alapuló gyorsmódszer Campylobacter jejuni hőpusztulásának vizsgálatára**. Magyar Állatorvosok Lapja 132, 47-53., 2010. *(IF: 0,088)*

Reichart O., Szakmár K., Jozwiak Á., Felföldi J., Baranyai L.: **Redox potential measurement as a rapid method for microbiological testing and its validation for coliform determination**. International Journal of Food Microbiology, volume 114, issue 2, 143-148., 2007. *(IF: 2,581)*

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