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CAMPYLOBACTER AND SALMONELLA SPP. IN REFRIGERATED BROILER MEAT

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Keywords: *Campylobacter*, *Salmonella*, poultry-meat

Introduction:

Poultry and poultry-meat serve as potential reservoirs for the transmission of pathogenic organisms, including *Salmonella* and *Campylobacter*. For years, changing nutritional habits have been leading to an elevation of the position of poultry meat. At the same time, this has resulted in an increased risk for the consumer. There are differing reports on the contamination of slaughter poultry with *Salmonella*. Investigations were carried out most frequently on the skin surfaces, musculature, caecum, liver, the skin of the cloacal area, and thawed water from frozen carcasses. In several European countries in humans, findings of *Campylobacter spp.* pathogenic for humans were more frequent than those of *Salmonella*. TOKUMARU et al. (1991) report that in Japan *Campylobacter* was found more frequently in poultry than *Salmonella* during the slaughter and cutting processes. On the other hand, in Germany, campylobacteriosis is the second most frequent cause of gastrointestinal infections.

Material and methods:

1. *Samonella:* In this study, 1008 fresh poultry samples (breast musculature, upper and lower leg musculature, skin) were investigated for the presence of *Salmonella*. The isolation and identification of the *Salmonella* was done with standard procedures.

2. *Campylobacter* - phenotypical detection: Culturing was done by use of the *Campylobacter*-Enrichment-Broth No. 2 (Oxoid No. CM 67) and Preston *Campylobacter* Selective Agar (PA, Oxoid CM 689) each of which was supplemented with 5% (v/v) saponine-lysed horse blood and *Campylobacter* Selective Supplement (Oxoid No. SR + 117). The samples in *Campylobacter* Enrichment Bouillon were incubated for 24h / 37°C and 24h /41°C; the culture was streaked onto a PA plate which was incubated for 48h at 25°C, 37°C and 42°C in a micro-aerobic enviroment.

3. Detection of campylobacter using RFLP-PCR: The following primers were used: CF03; CF02; CF02D; CF04 (Wegmüller et al., 1993). Optimized concentrations for PCR (100 µm total volume) were: 4mM $MgCl_2$,0.5 µM primers, 200 µM each of daTP, dCTP and dTTP (Promega, Madison, WI U.S.A.), 1 x reaction buffer (Promega), 0.02% BSA, 2 units of Taq DNA polymerase (Promega). Samples were covered with 80 µl mineral oil (Sigma, St. Louis, MO U.S.A.) and subjected to PCR in a PHC-3 thermal block (Techne, Princeton, NJ U.S.A.).

Results:

Results of *Salmonella* and *Campylobacter* detection in fresh poultry meat with conventional, i.e. culture methods, are shown in Tab. 1. Of 1008 investigated samples, a total of 382 samples (37.8%) were positive for *Salmonella*, and a total of 401 samples (39.7%) were *Campylobacter* positive. It can be observed that in fresh poultry meat, *Campylobacter* is prevalent over *Salmonella*. Tab. 2 shows the results of *Campylobacter* detection in fresh poultry meat using traditional culture methods compared to the RFLP-PCR. In the investigated 1008 samples, between 27.9% and 41.5% were positive in the culture methods, and between 31.0% and

47.6% were positive in the RFLP-PCR. The detection rate of *Campylobacter* using molecular biological methods is thus 3.1% to 6.5% higher than with the culture methods.

Tab. 1 Salmonella and Campylobacter in poultry meat

No. of investigated samples (per month)	Salmonella positive %							Campylobacter positive %						
	VI	VII	VIII	IX	X	XI	XII	VI	VII	VIII	IX	X	XI	XII
168	38.1	34.5	25.5	41.6	32.7	30.3	24.4	27.9	31.5	32.7	29.1	37.4	41.5	38.4

Tab. 2 Detection of Campylobacter using culture methods and RFLP-PCR

No. of investigated samples (per month)	Salmonella positive %							Campylobacter positive %						
	VI	VII	VIII	IX	X	XI	XII	VI	VII	VIII	IX	X	XI	XII
168	27.9	31.5	32.7	29.1	37.4	41.5	38.4	31.0	35.2	35.8	32.8	37.0	47.6	44.5

Discussion:

Several culture methods for the isolation of *Salmonella* and *Campylobacter* from animal-derived products were tested for their suitability for detecting the pathogens in fresh poultry meat. These were well-established, standardised methods, which were used either in the prescribed way or otherwise modified in order to achieve better results. The detection of bacteria in foodstuffs using PCR has been successful in many cases (Gandrian, 1995). In such investigations, the DNA analysis is largely a replacement for the biochemical or serological identification of the bacteria. A certain gene combination is of particular interest for the selection of the final sequence for the definition of a PCR system for the detection of *Campylobacter spp.* in meat or foodstuffs of animal origin: *Campylobacter* contains two genes (flaA and flaB) which are coded for two flagellin proteins. Information on these two genes as well as on defined PCR primers permit the direct detection of *Campylobacter spp.*. In Germany, *Salmonella* are the most important cause of human enteritis, where as in Switzerland and other EU-countries, *Campylobacter* has been most important. The rate of *Salmonella*-positive samples of 25.5% in August and 41.5% in September is alarmingly high. There were more positive findings in musculature samples than in skin samples. This is the result of secondary contamination of the investigated meat. The samples that we investigated for *Campylobacter* arrived at the laboratory in a frozen state, which is of major importance for the detection rate, because of the sensitivity of *Campylobacter spp.* In 27.9% to 41.5% of cases, *Campylobacter* was detected using culture methods, and up to 47.6% using RFLP-PCR. The results of our investigations show that the combination of culture methods and molecularbiological methods allows a better rate detection.

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