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Detection of *Salmonella* in poultry meat using culture method, enzyme-linked fluorescent immunoassay and immunochromatography

Nachweis von Salmonella in Geflügelfleisch mittels kultureller Methoden, Immunoassay und Immunchromatographie

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Summary

Three different methods (culture, enzyme-linked fluorescent immunoassay [ELFA] and immunochromatography) were compared for the detection of *Salmonella* spp. in 60 poultry meat samples. The number of salmonellae needed to give a positive reaction in the detection step of the NMKL, the VIDAS® SLM and the Singlepath® Salmonella was 10^2, 10^5 and 10^7 cfu/ml, respectively. The number of *Salmonella*-positive samples by culture and ELFA were higher (8 %) compared with those obtained by immunochromatography (2 %). The *Salmonella* contamination rate of turkey meat (19 %) was significantly ($p< 0.05$) higher than that of chicken meat (3 %). Three serotypes (*S. Typhimurium, S. Blockley* and the monophasic *S.* 6,8:e,h:-) were identified. When compared with the ELFA and the laborious and time-consuming culture method, the immunochromatography (lateral flow) proved to be the most user-friendly as no technical experience was required, however, the sensitivity was low. Both the ELFA and the immunochromatography provided rapid results.

Keywords: chicken, turkey, ELISA

Zusammenfassung

Für den Nachweis von *Salmonella* aus Geflügelfleischproben (n = 60) wurden drei unterschiedliche Methoden (Kultur, Immunoassay [ELFA] und Immunchromatographie) miteinander verglichen. Der Nachweis von *Salmonella* war mittels kulturellem Verfahren und ELFA höher (8 %) im Vergleich zur Immunchromatographie (2 %). Putenfleisch (19 %) war signifikant höher ($p <0,05$) mit Salmonellen belastet als Hähnchenfleisch (3 %). Drei Serovaren (*S. Typhimurium, S. Blockley* und die monophasische *S.* 6,8: e, h :-) wurden identifiziert. Verglichen mit dem ELFA und dem zeitaufwendigen kulturellem Verfahren erwies sich die Immunchromatographie als äußerst benutzerfreundlich, da keine technische Erfahrung erforderlich war, jedoch war die Sensitivität gering. Sowohl der ELFA als auch die Immunchromatographie lieferten schnelle Ergebnisse.

Schlüsselwörter: Hähnchen, Puten, ELISA

Introduction

Salmonella is one of the most commonly reported causes of human gastroenteritis in the European Union (EU). Poultry products, particularly fresh poultry meat are often implicated in human salmonellosis cases and they are the most frequently reported cause of foodborne outbreaks in the EU. The detection of *Salmonella* spp. is therefore an important microbiological parameter to assure food safety.

Microbiological analyses of *Salmonella* spp. using conventional culture methods are very time-consuming and completion requires five days or longer, making them inappropriate for the routine testing of a large number of samples. A number of less laborious and less time-consuming immunological methods have been used as an alternative to culture methods for the detection of *Salmonella.*

The VIDAS system is an automated enzyme-linked fluorescent immunoassay (ELFA) that uses specific *Salmonella* antibodies coated on the inner surface of the reagent strip. This system, which is validated by the Association Française de Normalisation (AFNOR), enables a rapid screening and a high throughput of samples for the detection of *Salmonella.* The Singlepath® *Salmonella* method is an immunochromatographic (lateral flow) test based on gold-labelled antibodies. Both methods reduce the screening time to less than two days.

Previous studies reported on a comparison of the VIDAS® SLM with culture, but there is only limited research on comparisons to and the usefulness of the Singlepath® *Salmonella.* The aim of this study was to compare two rapid methods with the culture method for the detection of *Salmonella* spp. in poultry meat samples in light of their applicability in the routine screening of food samples.

Materials and methods

Sample collection

A total of 60 naturally contaminated raw poultry samples, including chicken (n = 39) and turkey (n = 21) meat, were collected at the retail market in Munich, Germany between July 2010 and February 2011. Refrigerated samples were transported to the laboratory and analysed within 24 h.

Determination of the detection limits of the methods

The detection limit of all three methods was determined in two runs (*S. Typhimurium* was used in the first run and *S. Enteritidis* in the second run). *Salmonella*-negative poultry meat samples were analysed following the protocol of each method. Serial *Salmonella*-dilutions containing numbers of *Salmonella,* from 10^8 to 10^0 cfu/ml, were transferred just before screening into the last broth. They were then analysed according to the three methods: 18 analyses using M-broth for the ELFA method and 36 analyses using Rappaport-Vassiliadis (RVS) broth for the NMKL and the Singlepath® *Salmonella* method. Simultaneously each *Salmonella*-dilution was plated onto two chromogenic agars to determine the cfu/ml. One uninoculated sample was included as a negative control.

Detection of *Salmonella* spp.

Pre-enrichment step/sample preparation

A 25 g sample was pre-enriched in 225 ml buffered peptone water (BPW) (Merck, Darmstadt, Germany) and incubated for 16–20 h at 37 °C. This enrichment was used for the testing of all three methods. In a second enrichment step, 0.1 ml BPW was transferred to 10 ml RVS broth (Merck). The same RVS broth was used for all three methods, it was incubated for 6–8 h at 42 °C for the VIDAS® SLM (bioMérieux, Marcy L'Etoile, France) method and then for another 18–20 h at 42 °C for culture and the Singlepath® Salmonella (Merck) method. After enrichment in RVS, samples were screened for *Salmonella* spp. using all three methods. Additionally, presumptive positive results of the VIDAS® SLM and the Singlepath® were confirmed by culture.

Culture method

The culture method for *Salmonella* spp. was performed in accordance to the Nordic Committee on Food Analysis (NMKL) and was considered the reference method. One loop (10 µl) of the RVS broth, which had been incubated overnight, was streaked onto Xylose Lysine Deoxycholate (XLD, Merck) and Rambach agar (Merck). The plates were incubated for 18–24 h at 37 °C. Presumptive *Salmonella* colonies were subcultured onto Plate Count agar (Merck). Identification was performed using the Enterotube (Becton Dickinson GmbH, Heidelberg, Germany) and *Salmonella* omnivalent sera (Siemens Healthcare, Marburg, Germany). From each positive sample five *Salmonella* colonies, isolated either from XLD and/or Rambach agar, were sent to the Bavarian Health and Food Safety Authority in Oberschleißheim, Germany for serotyping according to the Kauffmann-White scheme by agglutination with specific antisera (Sifin, Berlin, Germany). Using the NMKL method, negative or presumptive positive results are obtained not earlier than 3 days after initiation of the test.

ELFA method

For the detection of *Salmonella* spp. with ELFA, the VIDAS® SLM method was used. There are several enrichment protocols available for the detection of *Salmonella* using the VIDAS® system. In the present study the RVS and M-broth were used because the aim was to compare methods that basically used the same enrichment protocols. After incubation of the RVS broth for 6–8 h at 42 °C, 1 ml was transferred to 9 ml M-broth (bioMérieux) followed by incubation for 16–20 h at 42 °C. Subsequently, 1 ml of M-broth was boiled for 15 min, then 0.5 ml was transferred to the VIDAS® SLM strip (bioMérieux) and analysed according to the manufacturer's instructions. Samples showing a test value (TV) of ≥ 0.23 were considered presumptive positive, as indicated by the manufacturer. If results were equal to or above the threshold of 0.23, confirmation was achieved by streaking one loop of the non-heated RVS and M-broth onto XLD and Rambach agar. Further processing was performed according to the NMKL method described above. Using the VIDAS® SLM with this enrichment protocol, negative or presumptive positive results are obtained no sooner than 2 days after initiation of the test.

Immunochromatography

The Singlepath® Salmonella was used to detect *Salmonella* spp. by the immunochromatographic method. After the RVS broth was incubated 24 h at 42 °C, 1 ml was boiled, cooled to room temperature and 0.16 ml was transferred to the nitrocellulose membrane. According to the manufacturer, in the case of positive results two different red lines would appear within 20 min. Yet only one red line was observed when testing negative samples. To confirm presumptive

Journal of Food Safety and Food Quality 64, Heft 1 (2013), Seiten 1–32

positive results, one loop of the unboiled RVS broth was streaked onto XLD and Rambach agar and processed according to the culture method described above. The Singlepath® Salmonella gives negative or presumptive positive results not earlier than 2 days after initiation of the test.

Statistical analysis

The x^2 (chi) test according to McNemar was used for statistical analyses in order to evaluate the differences between the number of positive results in the groups. For all comparisons, the significance level was considered $\alpha = 0.05$.

Results and discussion

The number of salmonellae needed to give a positive reaction in the detection step of the NMKL, the VIDAS® SLM and the Singlepath® Salmonella was 10^2, 10^5 and 10^7 cfu/ml, respectively. These results were comparable with those described for the VIDAS by Blackburn et al. and by Becker et al. The detection limit of the Singlepath® Salmonella was high, but was still within the range of 10^4 to 10^7 cfu/ml given by the manufacturer. As expected, the culture method was shown to be the most sensitive method for *Salmonella* detection.

The culture method was found to be the most laborious and time-consuming. Its major disadvantage is known to be the generation of presumptive false-positive results due to the similar colony appearance of some strains, especially *Citrobacter freundii.* This problem was not observed in the present study. All presumptive positive results obtained by the VIDAS® SLM and Singlepath® could be confirmed by culture. The frequencies of *Salmonella*-positive samples tested by NMKL and the VIDAS® SLM were higher when compared with those obtained by Singlepath® *Salmonella* (Tab. 1). Similar results were found by Korsak et al. and Eriksson and Aspan. Both reported that the VIDAS® was comparable with the NMKL method. In contrast, Reiter et al. found twice as many positive samples when using the VIDAS® SLM as opposed to the culture method, but in the latter two methods different selective agars and selective enrichments were used. The NMKL and the VIDAS® SLM methods presented the best performance with a 100 % agreement. The high sensitivity (100 %) and specificity (100 %) observed in the present study for the VIDAS® SLM has also been observed before.

In comparison, using the Singlepath® Salmonella, four false-negative results were obtained. It is noteworthy that two samples (one with serotype 6,8:e,h:- and one with serotype Blockley) formed a slight red line after 24 h, but these results are not valid since they were not read within 20 min. after test initiation. To prevent influences of the enrichment on the detection of *Salmonella,* the same selective enrichment was used in the present study. Thus, false-negative results by the Singlepath® Salmonella seem to be generated by the detection method itself, probably because the *Salmonella* concentration was too low. The lower concentration of salmonellae might be explained by the slower growth of certain serotypes. Another possibility would be that the Singlepath® Salmonella gave false-negative results for *S.* 6,8:e,h:- and *S. Blockley* because of a lower binding capacity of the antibodies to these serotypes. To find out which of the two possibilities had led to the false-negative results, we re-examined all *Salmonella* isolates; each *Salmonella*-isolate was transferred into BPW and treated like a sample until screening. In addition, 0.1 ml of the RVS and M-broth were plated onto XLD and Plate Count agar in order to determine the cfu/ml. This time the Singlepath® detected all *Salmonella*-serotypes. However, serotype 6,8:e,h:- only formed a very slight red line after 20 min. The cfu/ml of S. 6,8:e,h:- varied between 10^6 and 10^7 while the cfu/ml of the other serotypes were higher than 10^7. These results suggest that the false-negatives obtained by the Singlepath® were not dependent on the serotype but on the concentration. To guarantee the consumer's health, it is necessary to apply methods that restrict the number of false-negative results.

The primary focus of this study was on the comparison of methods and it was not intended to provide statistically relevant data on the occurrence of *salmonella* in poultry meat. Nevertheless, it is interestingly to mention that the overall contamination rate of raw poultry (8 %) was lower than reported in previous studies worldwide, but it was similar to the 7 % found in broiler meat in Germany in 2008. Turkey meat (19 %) was significantly ($p< 0.05$) higher contaminated with *Salmonella* spp. in comparison to chicken meat (3%). This finding is different from most previous reports where chicken meat rather than turkey meat has been shown to be more frequently contaminated with *Salmonella.*

A total of 25 *Salmonella* isolates from 5 samples were obtained. The samples were always contaminated with only one serotype. Three different serotypes were obtained, the most common (n = 15) was serotype 6,8:e,h:- (Tab. 2) which is a monophasic variant of *S. enterica* serotype Newport (antigenic formula

TABLE 1: *Comparison between the results obtained with the Singlepath® Salmonella (SP), the VIDAS® SLM and the NMKL (reference method) in 60 naturally contaminated poultry meat samples.*

	SP vs. NMKL			VIDAS® SLM vs. NMKL		
	SP positive	SP negative	Total	VIDAS® SLM positive	VIDAS® SLM negative	Total
NMKL positive	1	4	5	5	0	60
NMKL negative	0	55	55	0	55	55
Total	1	59	60	5	55	60

TABLE 2: *The detection rates of Salmonella spp. and Salmonella serotypes found in raw poultry meat samples.*

Sample type	No. of samples	No. of positives (%)	No. of positive samples via: NMKL	VIDAS®	Singlepath®	Serotype	Antigen-formula
Chicken	39	1 (3)	1	1	1	*S. Typhimurium*	4,5: i:1,2
Turkey	21	4 (19)	4	4	0	*S. Blockley,* *S.* 6,8:e,h:-	6,8:k:5 6,8:e,h:-
Total	60	5 (8)	5	5	1		

6,8:e,h:1,2). *Salmonella* Newport is a serotype commonly isolated from cattle and humans but it has also been sporadically found in poultry. Our findings indicate that turkey meat might be a possible source of the monophasic *S.* Newport.

In conclusion, turkey meat was higher contaminated with *Salmonella* than chicken meat. The monophasic *S.* 6,8:e,h:- was the most common serotype detected. Both the ELFA and the immunochromatography can be an alternative method for culture, especially when rapid results are needed. Due to its automation, the VIDAS® assay facilitates the correct interpretation of results and allows for comparison between results from different laboratories. The performance of the Singlepath® Salmonella did not require technical experience and it was a rapid and user-friendly screening method, however, the sensitivity of the assay was low. Results of the Singlepath® Salmonella did vary, depending on the *Salmonella* concentration. The culture method was shown to be the most time consuming and laborious, especially for the screening of negative samples. Nevertheless, culture is necessary to confirm presumptive positive results of the other two methods and it is the only one of the three methods which is able to detect viable bacteria.

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