THE OCCURENCE OF SALMONELLA IN A POULTRY ABATTOIR AND CUTTING-UP ENTERPRISE AND THEIR DIFFERENTIATION BY MEANS OF PLASMID ANALYSIS

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Since the middle of the 80s there has been a clear increase in several European states in the number of intestinal infections caused by Salmonella bacteria in Man. In Germany the number of Enteritis caused by Salmonella increased dramatically from 1986 to 1992. From 1993 a small decrease has set itself up in contrast to this global growth in Salmonellosis. The occurence of Salmonellosis in Man is primarily caused by contaminated Foodstuffs. Correspondingly, a large number of Salmonella serotypes has established itself in the animal populations kept for the winning of foodstuffs. Thus a large number of Salmonella-Serovars have been detected, for example in poultry. Here a change is to be noticed since the middle of the 80s, coupled with a large increase in S. enteritidis. This development also occured on other continents at almost the same time, though regional differences in the Phangentypes have appeared. In Europe the S. enteritidis Phangentype 4 is the most prevalent, the position is different in the USA where Phangentypes 8 and 13a are dominant. S. enteritidis has been discovered to be the causer in 90 % of the Salmonellosis associated with foodstuffs in Man. S. enteritidis has been discovered to be dominant in the case of poultry too and has been isolated with a large distance from S. virchow, S. livingstone, S. saint paul, S. agona and S. bredeney and other Salmonella serotypes. Contrary to the position with Man, however, in the last two years, S. hadar has become the dominant serovar in poultry.

Materials and Methods

Salmonella-Isolates

Within an investigation time of 6 month 2280 poultry-flesh specimes have been taken in a Poultry abattoir and its Cutting-Up Enterprise in North West Germany from the skin, the musculature and inner organs, and these have been examined for Salmonella.

The isolation of Salmonella occured with the following methods:
- Pre-enrichment with Peptone water
- Selective enrichment with the Rappaport-Vassiliadis-Medium
- Selective Agar plates: Rambach Agar and XLD-Agar

After this the Salmonella Isolates were differentiated biochemically and serologically. 175 Salmonella strains were molecularbiologically further differentiated in accordance with serotyping for plasmid profile.

Plasmid-Isolation

Palsmid-DNA was isolated out of 2 ml Culture LM-Medium, obtained by means of incubation at 37 °C overnight in accordance with the modified method of KADO and LIU (1981). The electrophoresis of the Plasmid-DNA was carried out with 0,8 % horizontal Agarose gel in a TBE buffer and a consequent colouring with Ethidiumbromides.

Results and Discussion

A differentiation takes place on poultry between the infection with the host adapted Serovar S. gallinarum-pullorum and other Serovars (s. hadar, S. infantis, S. saint paul, S. arizonae).
S. enteritidis has spread in the poultry stocks since the middle of the 80s and was until 1993 the most frequently isolated serovar. Since then, at least in northern Germany, a serovar change has appeared, in that 7 % of all Salmonella Isolates could be identified by us as S. hadar. It is possible to see the frequency of all Salmonella-serovars isolated from chicken-flesh in 1995 in Table 1.
The decline in of S. enteritidis is possibly the expression of an antibiotic therapy or of a vaccination of the aminals in their housing. That does not mean, however, that S. enteritidis has already been eliminated from the environment of the animals or from poultry-flesh.
The increase occurence of S. hadar at the time of the slaughtering os not surprising. S. hadar was also dominant within the framework of our investigation with one day old chicks and animals of different life ages. Here it is apparent that despite the occurence of S. hadar in flesh this serovar could not be diagnosed in the organs of the slaughtered chicken. However, S. enteritidis was isolated in the organs (liver and cecum). Numerous factors, such as treatment, virulence differences or other additional bacterial or, as the case may be, virus-caused illnesses as well as stress, appear to have played a role. During our investigations 5 non host-specific adaptive Salmonella were frequently isolated: S. hadar, S. virchow, S. enteritidis, S. indiana and S. blockley. Further to this 5 % of all isolated Salmonella belonged to the S. livingstone serovar.
The increased appearance of S. livingstone within the poultry trade area should be expected in the future.
The results of this work follow the discoveries of Barrow (1993), whereby the occurence of Salmonella in the highly industrialised countries is only determined through a few serovars. The importance of these serovars as the causers of relevant illnesses from the point of view of foodstuffs-hygiene have been described by HARTUNG as regards S. hadar (1993), and by BARROW as regards S. hadar and S. virchow (1993) and by SELBITZ as regards S. indiana (1995).
Especially most recently, intensive work has been carried out to improve the microbiological Salmonella diagnostics and to reduce the length of time to the examination. Here a more specific differentiation method is of particular importance in the hygiene of foodstuffs. Routine microbiological investigations, including the biochemical and serological diagnostics last to 6 days. After this time it is only possible to make a statement concerning the total number of germs and their serological classification. The work which has been admitted confirms the possibility of differentiating representatives of the same and of different Serovars more finely and of compiling an epidemiological study of Salmonella of different Serovars with the help of the plasmid profile analysis.
S. enteritidis is specially alarming within the poultry trade because the higher virulence of this Serovar often leeads to human

Salmonellosis. In the Period of Time on hand for the Examination 11 % of S. enteritidis strains were isolated. 96 % of these Isolates contain a 37 Md Pladmid. This Plasmid is serovar-specific and the suspender of virulence characteristics (Atanassova, V., 1993).
S. hadar is the Salmonella serovar which is most frequently isolated. 72 % of all S. hadar strains examined possess a Plasm. S. hadar Isolates suspend a 34 Md Plasmid and in most cases smaller Plasmids too - 5,8; 2,2; 1,8 and 1,0 Md. Whether the 34 Md Plasmid in S. hadar is combined as a Gene carrier for antibiotic resistances and/or other virulence factors, remains questionable. The results of the resistance tests show that most of the S. hadar strains tested reveal no resistance towards the substances employed.
The plasmid profile analysis has the advantage of demarcating homogeneous and irregular strains of the Salmonella Isolate from each other.

Table 1: **Frequency of individual Salmonella Serovars**

Salmonella Serovar	Number	in %	with Plasmid	without Plasmid
S. hadar	36	27,3	72	28
S. virchow	24	18,1	100	-
S. eneritidis	23	10,0	96	4
S. indiana	13	9,8	39	61
S. blockley	9	6,8	78	22
S. infantis	7	5,3	14	86

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