8. Summary

Experiences with the vaccination of laying hens in alternative husbandry with different inactivated *E. coli* vaccines under field conditions

Diseases caused by *E. coli* are numbered among the most frequent causes of increased mortality rates in the alternative husbandry of laying hens. Due to the very limited therapeutic possibilities for laying hens, prophylactic measures such as vaccinations are of great significance.

Within the framework of this study, various population-specific inactivated *E. coli* vaccines were tested on 7.196 laying hens living in alternative husbandry systems. The laying hens were divided into four test groups and one unvaccinated control group, and stayed, for both the breeding and the laying period, under equal conditions in the same coop. The strains used for the population-specific vaccines were isolated from laying hens which had died from colibacteriosis, then serotyped and with the aid of PCR checked for the presence of 7 APEC specific, virulence associated genes (*astA, irp-2, fyua, iucD, tsh, fim C, pap C*). After carrying out a pathogenicity test, we eventually selected 3 out of 25 isolated *E. coli* strains in the embryonated egg for the vaccine production, two of them being isolates of the O-group O1 and one of them an isolate of the O-group O18. They showed 5, 6 or 7 of the virulence associated genes. Two vaccines were produced from it which contained all three strains, but differed in their amount of germs (10^8 resp. 10^9 KbE per ml). These vaccines were subcutaneously administered to the animals on one or two occations during their breeding. The measurement of results of the examined vaccines was carried out based on the clinic examination of the animals, on the mortality rate and pathological-anatomical examinations as well as on bacteriological and serological tests which were performed using the indirect ELISA test system developed by ourselves. Clinically, there were no variations from the state of health and the general condition to be expected for animals of that age. The mortality rate during the laying period averaged at 7.38 %, while the values ranged from 6.54 % (unvaccinated control group) to 8.35 % (one vaccination with 0.5 ml x 10^9 KbE/ml during the 12th laying week). In the course of the whole study, we could not determine any differences between the vaccinated groups and the unvaccinated control group regarding the clinical parameters and the mortality. Bacteriologically, 36 strains of *E. coli* could be isolated, 21 of which were serotyped and tested for virulence associated genes. While there were only 4 (19.0 %) *E. coli* isolates that could be allocated to serotype O2 through serotyping, we were able to detect at least one APEC specific, virulence associated gene [(*iss* (95,2 %), *iucD* (52,4 %), *irp2* (33,3 %), *astA* (33,3 %), *tsh* (23,8 %), *papC* (4,8 %)] in all tested *E. coli* strains using the molecular biological detection method. In particular the high prevalence of the anti-

host defence system Iss (incresaed serum survival; *iss*: 95,2%) not covered by the vaccine points out its key role in the pathogenesis of colibacteriosis.

The serological progression study showed an increase of the serum titer until the 19th laying week (LW), whereas the comparison of the mean values from the vaccine groups' measurement readings of the 12th and 16th LW (Scheffe's procedure within the multicomparison testing) presented a significant difference ($P \leq 0.05$) of the increase of titers between the group of two vaccinations (2 x 0.5 ml x 10^9 KbE/ml) and the unvaccinated control group. From the 20th LW onwards, the antibody level began to fall steadily and in the 50th LW, it showed its lowest value of the whole laying period. For the diagnostic serological test of hen serums, we demonstrated the specifity of the applied indirect ELISA for the detection of antibodies against *E. coli* and proved the repeatability of the results with intra- and interassay differences.

Towards the end of the laying period, a load test was conducted on 50 animals per group. For this purpose, 25 animals per group were intratracheally administered 0.5 ml of a germ-containing Tryptose Phosphate Broth (10^7 KbE resp. 10^8 KbE). The strained animals were observed and the time sequence of their mortality documented. The results of this test indicated that groups with a higher challenge dose also showed higher mortality rates regardless of the respective test group. The mortality rate of the groups with the higher infection dose ranged between 68-80 %, while the lower infection dose ranged between 48 and 68 %. Therefore, we cannot assume that the *E. coli* vaccination has an effect on the course of mortality or that there is an immunization until the end of the laying period of the hens.

As a conclusion of this study, there was no measurable effect of the *E. coli* vaccination on the development of productive relevant parameters such as the mortality of laying hens within one group and between the test groups. If we take, according to the usual practice, the health of the hen population as a basis for the control of success, there is a definitely positive tendency throughout the development of the overall mortality (7.38 %) compared to the rate of previous herds (25-30 %). However, the employment of population-specific *E. coli* vaccines can only be a promising remedy for controlling APEC infections if sufficient pathogen identification tests, especially of the present virulence factors, are conducted beforehand. For an efficient prophylaxis, it is not only indispensable to ensure proper application of effective *E. coli* vaccines, but also to optimize all management parameters (husbandry, feeding and biosecurity).