DYNAMIC OF EXCRETION AND IMMUNE RESPONSE OF EXPERIMENTALLY INFECTED PIGS WITH MONOPHASIC VARIANT OF SALMONELLA TYPHIMURIUM SEROVAR 1,4[5], 12:i:-

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Introduction
In recent years Salmonella enterica serovar Typhimurium 1,4[5],12:i:- (monophasic variant of S. Typhimurium or vmST) was focused as a major zoonotic problem because produce human gastroenteritis outbreaks in many countries. Human disease caused by this serovar is considered as emergent in European Union countries and actually is ranked as the third most reported serotype after S. Enteritidis and S. Typhimurium (EFSA, 2016; Fernandes et al., 2016).

Swine (pig and pork products) was reported as reservoir of vmST. In pig production systems, Salmonella colonization in the pig intestine, and subsequent excretion, determine the possible contamination of the meat for human consumption. Extensive information about colonization and immune response in pigs are available for S. Typhimurium but not for vmST.

Thus, the aim of this work was to study the dynamic of excretion and immunological response of pigs after inoculation with a vmST, using an experimental Specific Pathogen Free (SPF) pig model. Excretion was followed twice a week by numeration of Salmonella. Moreover, from blood serum, the antibody presence was measured by ELISA assay to assess the humoral response in pigs (Osterberg & Wallgren, 2008) and the mesure of interferon Gamma (INF-γ) was used as marker of innate immunity for Salmonella infection (Huang et al., 2011; Uthe et al., 2009).

Materials and methods
The trial was conducted on 32 SPF piglets, born into ANSES Ploufragan protected animal house. Piglets aged of 5 weeks were placed in hermetic and separated experimental animal houses. Inoculated experimental herd of 24 piglets was placed in three separate rooms (8 piglets per room), each one containing 2 pens. Four piglets were placed in each pen. The three groups of 8 pigs (G1, G2 and G3) were slaughtered at different ages, were followed respectively during 21, 49 and 84 days post inoculation (p.i.). Control herd of 8 pigs was kept in 2 separate rooms.
Pigs were monitored daily for surveillance of clinical manifestations and body temperature control. Food consumption and body weight were reported weekly.

A field strain of vmST was used for the experiment. In order to easily enumerate the strain in fecal samples, it was transformed in strain resistant to Rifampicin (SRif). At seven weeks of age, piglets were orally inoculated with 10 ml of a solution containing a SRif bacterial solution of $10^8$ CFU per ml. Control herd received only 10 ml of Tryptone Salt Broth. Fecal samples were taken twice a week, directly from the animal rectum. Blood samples were taken from the jugular vein once a week, beginning at day 7 p.i. for G3 and at day 3 p.i. for G1 and G2.

Fecal samples were diluted 1:10 with Buffered Peptone Water (BPW). 5 ml of this dilution ($10^{-1}$) were taken and serials dilutions were performed in Salt Tryptone Broth tubes until dilution $10^{-4}$. 1 ml of $10^{-1}$ dilution was seeded in 3 plates of Xylose Lysine Desoxycholate agar supplemented with Rifampicin (XLD+Rif). 100 µl of dilutions $10^{-2}$ and $10^{-4}$ were also plated in XLD+ Rif. Plates were incubated at 37 °C for 24 hours. Typical black colonies of *Salmonella* were counted and expressed in Log10 CFU/g. When enumeration was negative, a *Salmonella* detection protocol was performed following NF-U47-102.

For antibody screening, IDEXX Swine *Salmonella* Ab Test® (IDDEXX, Montpellier, France) was used. Samples with OD% values equal to or greater than 15% (S/P=0.375) were considered as positive.

Interferon γ response was measured in serum samples, using Porcine IFN γ ELISA Kit ® (Thermo Fisher Scientific, Villebon-sur-Yvette, France), according to the manufacturer instruction. Results were expressed in pg/ml and they were compared with LLD (Lower Limit of Detection) calculated for each plaque.

Pigs were euthanized and autopsied at 21 days (8 inoculated pigs and 2 controls), at 49 days (8 inoculated pigs and 2 controls) and 84 days (8 inoculated pigs and 4 controls). We took in aseptic conditions tonsils, mesenteric lymph nodes, and different parts of the intestinal content: duodenum, jejunum, ileum and caecum. All samples were analyzed following the microbiological protocol describe above for the feces.

**Statistical analysis**

To establish the excretion kinetic we compared the excretion levels of pigs each day after inoculation (24 pigs during 21 days, 16 pigs during 49 days and 8 pigs during 84 days). Area Under Curve (AUC) was measured for individual excretion for all pigs until day 21 post inoculation (p.i.). AUC obtained were compared with non-parametric method (Kruskal-Wallis test). Pearson’s correlations (p<0.05) were performed between the results of pigs excretion and the antibody and interferon response.

**Results**

**Excretion in feces**

All groups of inoculated pigs had shed vmST continuously during respectively 21, 49 and 84 days p.i. with daily variations of excretion (Figure 1).
Figure 1. Blox pot representing the excretion kinetic for all pigs during 84 days (in Log10 CFU/g).

After a peak of excretion just post inoculation (5.8 ± 1.6 log10 CFU / g at day 3), the mean amount excreted decreased significantly (2.4 log 10CFU/g at day 7). The lower amount was detected at day 53 (1.4 ± 0.7 log10 CFU / g).

Comparison of AUC (Area under curve) measured for each pigs using Kruskal-Wallis test did not show a significant difference between the 32 pigs (p> 0.05) for excretion in feces from day 0 to day 21. Inside G1 and G2 we did not find significant differences between pigs (p=>0.05). However in G3, we found significant difference in pig’s excretion levels (p<0.001). Control group remained negative for Salmonella during all the trial.

**Immune response**

**Kinetics of individual seroconversion**

Antibody response started at day 7 p.i. with variability among experimental groups and pigs. 100% of pigs from Group 2 and 3 were seroconverted at day 49 p.i. and remained positives until the end of experiment (Figure 2). A positive correlation was found between excretion of vmST in feces and serological results with Pearson test p=-0.29

Figure 2. Frequency (%) of seropositive pigs during 84 days.
Interferon γ level in serum

50% of pigs showed IFN γ levels higher than 50 pg/ml at day 3 p.i. (Figure 3). These levels decreased at day 7, and became lower than 15 pg/ml in 80% of pigs. At day 14, IFN γ were not detectable. Positive correlation between IFN γ and excretion levels was found at day 3.

![Figure 3. Interferon γ average and excretion levels in pigs artificially contaminated with Salmonella during 21 days. (Group 1 and 2).](image)

Conclusion

To our knowledge this work is the first description of excretion dynamic and immunological response of experimental pigs after infection with a monophasic variant of Salmonella Typhimurium strain. Fecal shedding of the 24 pigs used was persistent and continuous during 84 days. At the autopsies, the highest contamination was evidenced in tonsils and the lowest in mesenteric lymphatic nodes, for all pigs of the three experimental groups. Concerning antibody response, seroconversion begins at day 7, and all pigs followed during 49 and 84 days seroconverted at day 49 post-inoculation. The highest levels of INF-γ were highlighted 3 days after inoculation with the monophasic variant of S. Typhimurium.

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References


