Use of an avirulent live *Salmonella* Choleraesuis vaccine to reduce the prevalence of *Salmonella* carrier pigs at slaughter

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This study evaluated the use of an avirulent live *Salmonella* Choleraesuis vaccine to reduce the seroprevalence and number of *Salmonella* carrier pigs at slaughter. Seven batches of 500 pigs were included in each of the two study groups: the vaccinated group (VG) that was orally vaccinated and the control group (CG) that received a placebo on the first day of life. The groups were managed in a three-site system and followed up from birth to slaughter. Blood samples (n=378) were collected from each VG and CG to monitor the on-farm seroprevalence in both groups. Mesenteric lymph nodes and blood from animals (n=390) belonging to each group were collected at slaughter. At the first day of life, the seroprevalence in control batches ranged from 77.9 to 96.3 per cent, while in vaccinated batches, it ranged from 66.6 to 92.6 per cent. At weaning (21 days of age), the number of seropositives decreased in both groups (mean of 12 and 3.7 per cent for CG and VG, respectively). At slaughter, batches of VG had a significantly (P<0.0001) lower seroprevalence (46.6±5 per cent) and isolation of *Salmonella* from lymph nodes (33.1±5 per cent) compared with CG batches (79.7±4 per cent). The results indicate that administration of a *Salmonella* choleraesuis-attenuated vaccine on the first day of life decreases *Salmonella* isolation and seroprevalence in pigs at slaughter.
lent strain in swine neutrophils, resulting in the loss of the spv gene from the virulence plasmid (Kramer and others 1992, Roof and others 1992). Trials conducted on artificially inoculated mice and pigs demonstrated that the vaccine derived from SC-54 strain is safe and able to protect animals from infection and clinical disease (Kramer and others 1992, Baum and others 1997). Moreover, the use of this vaccine to control Salmonella carriers yielded promising results in trials conducted with artificially infected pigs (Letellier and others 2001), calves (Fox and others 1997) and dairy cows (House and others 2001).

In Brazil, studies have shown a high prevalence of carrier pigs at slaughter (Bessa and others 2004, Schwarz and others 2009), highlighting the fact that efficient and cost-effective measures, which can be associated with good management practices in farms, are needed to achieve Salmonella control. Thus, this study evaluated the use of the vaccine produced with the SC-54 strain in a herd with a high Salmonella prevalence with the aim of reducing the number of Salmonella carriers at slaughter.

Materials and methods

Location of the trial

The trial was conducted in a three-site vertical integration company with a history of a high prevalence of pigs carrying S enterica in the mesenteric lymph nodes (85 per cent) and a high rate of seropositivity (77.8 per cent). The most prevalent serovars in this herd were S Brandenburg, S Typhimurium and S Agona (Schwarz and others 2009).

Vaccine

The animals were vaccinated with the attenuated live vaccine EnterisISC-54 (Boehringer Ingelheim Vetmedica GmbH) containing the Salmonella Choleraesuis variety Kurzendorf SC-54 strain attenuated via several passages in pig neutrophils (Roof and others 1992).

Study design and management of the animals

A controlled, blinded clinical trial was designed to assess the efficacy of EnterisISC-54 orally administered to piglets. The experiment was started in the breeding site, located in a farm with a 1000 sow inventory housed in two barns. The facilities and management practices in both barns were similar. Sows were maintained in individual crates of life and iron dextran was routinely administered to animals of that carrier group. Piglets born to sows housed in one barn constituted the CG. Each group (VG and CG) included seven batches of 500 piglets born within a one-week interval over seven consecutive weeks, making a total of 3500 pigs in VG and CG. Both groups were managed according to the company’s standard practices in all three barns. Pigs not entered into the study were co-mingled with experimental carriers yielding promising results in trials conducted on artificially inoculated mice and pigs dem-
and individual lymph node (25 g) samples were processed using a *Salmonella* isolation protocol comprised of pre-enrichment in 1 per cent buffered peptone water, selective enrichment (tetraionate broth and Rappaport-Vassiliadis broth; Merck; 42°C, 24 hours) and isolation on solid medium (xylose-lactose-tergitol 4 agar and Brilliant Green-phenol red-lactose-sucrose agar; Bencton-Dickson and Company; 57°C, 24 hours), as previously described (ISO6579). Isolates identified as *Salmonella* species were shipped to Fundação Instituto Oswaldo Cruz for serotyping.

**Statistical analysis**

All statistical analyses were performed using commercial software (SAS 9.1.3, 2009). Logistic regression was used to test the relationship between vaccination and seroprevalence or *Salmonella* isolation from the mesenteric lymph nodes at slaughter. Ovdispersion was corrected by Williams’ method. p-Values <0.05 were considered significant. Spearman’s correlation was calculated between frequency of *Salmonella* presence in lymph nodes and the batch serology at slaughter.

**Results**

Seroprevalences ranging from 77.9 to 96.3 per cent were found in batches of one-day-old piglets belonging to the CG, while in vaccinated batches frequencies of seropositive piglets varied between 66.6 and 92.6 per cent. On day 21, a prominent decrease in the frequency of seropositives was observed in the batches of both groups (Table 1). No statistical difference between groups was observed in seroprevalence (P>0.05).

Five nursery facilities had environmental samples that were positive for *Salmonella* before the allocation of pigs, and both groups were exposed to a similar challenge (two of four positive farms in VG and three of four in CG). At 49 days of age, only one batch of VG had positive faecal samples. This group was housed in one of the farms that was positive on environmental sampling. All sampled animals from VG were serologically negative on this sampling event, while three batches of CG presented seropositive pigs in frequencies ranging from 3.7 to 5.5 per cent.

At the finishing phase (143-day-old pigs), most batches belonging to both experimental groups had at least one *Salmonella*-positive faecal pool. However, the number of positive pools was lower in batches of VG (median=1) compared with CG (median=3).

At slaughter, the isolation of *Salmonella* from mesenteric lymph nodes in VG (129/390; mean=33.1 per cent; IC95=28.1 to 38.1 per cent) was significantly (P=0.0001) less frequent than in CG (232/390; mean=59.5 per cent; IC95=54.5 to 64.5 per cent). The *Salmonella* serovars identified in the samples from both groups were similar, and S Agona (33 per cent), S Panama (27 per cent), S Ohio (11 per cent), S Schwarzenberg (11 per cent) and S Typhimurium (5 per cent) were the most prevalent. The seroprevalence at slaughter was also significantly (P=0.0001) lower in VG (174/390; mean=44.6 per cent; IC95=39.6 to 49.6 per cent) than in CG (311/390; mean=79.7 per cent; IC95=74.7 to 83.7 per cent). Vaccinated batches therefore had a lower chance of having pigs at slaughter that were positive for *Salmonella* isolation from lymph nodes (OR=0.33; CI95=0.19 to 0.57) and in ELISA testing (OR=0.20; CI95=0.09 to 0.42).

**Discussion**

This study demonstrated that the vaccine based on the attenuated strain SC-54 was able to significantly reduce the seroprevalence and the number of *Salmonella*-carrier pigs in mesenteric lymph nodes at slaughter. These results agree with those of previous studies where a reduction in the number of positive animals was found at slaughter, even though they adopted different vaccination protocols and evaluation parameters. The SC-45 strain was tested in previous studies that evaluated the administration of the vaccine via drinking water to pigs allocated in finishing farms (Baum and others 1997, Kolb and others 2002). In one trial, a statistically significant reduction in *Salmonella* isolation was achieved for serogroups B and C1 but not for serogroups C2 or E (Baum and others 1997). In the second trial, a significant (p=0.02) reduction in the frequency of *Salmonella* isolated from carcasses in the VG was observed in a herd that previously presented a prevalence of 26.2 per cent positive carcasses (Kolb and others 2003).

In our study, oral administration of the SC-54 strain vaccine to one-day-old piglets was evaluated in a vertically integrated system with a history of high (>70 per cent) seroprevalence and *Salmonella* isolation at slaughter. Vaccinated and control batches, following a longitudinal study, showed a similar pattern of transmission and seroconversion. One-day-old piglets of both groups presented a high seroprevalence, possibly caused by the transfer of maternal antibodies, as described by Funk and others (2001) and Chiu and others (2006). In this sense, piglets in both groups had been delivered by seropositive sows and both presented antibody concentrations that gradually decreased from birth until the 21st day of age.

The disappearance of colostral antibodies coincided with transfer to the nursery, where stress factors are responsible for increasing susceptibility to infection in pigs (Funk and others 2001, Fosse and others 2009). In addition, the residual environmental contamination of the nursery facilities observed in the study and the co-mingling with non-vaccinated pigs could have contributed to *Salmonella* transmission. Despite this, after 28 days (49-day-old pigs) in the nursery, only three batches were detected with a (low) prevalence of seropositive pigs (2 per cent, IC95=0 to 6 per cent) and only one cohort from the VG group was shedding *Salmonella*, indicating that most of the pigs were either negative for *Salmonella* or the animals were still in the initial stages of infection. At the finishing stage, the pigs started to shed *Salmonella* in their faeces, in line with the amplification in the number of infected animals often observed at this stage (Funk and others 2001, Lo Fo Wong and others 2004). However, the VG showed a lower number of positive faecal pools, which may have resulted from a lower number of infected animals due to the protection conferred by vaccination. Although a poor correlation between individual *Salmonella* ELISA tests and bacteriology on an individual pig basis has been reported (Christensen and others 1999, Davies and others 2003), the seroprevalence at slaughter has demonstrated a strong correlation with the presence of *Salmonella* in caecal contents and on the carcass surface in pig batches (Sørensen and others 2004). Thus, programmes to control *Salmonella* have aimed to reduce seroprevalence in herds to levels below 40 per cent, as well as the eradication of herds with isolates above 70 per cent in order to decrease the hazard of carcass contamination at slaughter (Mousing and others 1997, Alban and Ståk 2005).

In our study, all batches vaccinated with the SC-54 strain presented seroprevalences below 70 per cent, whereas the control cohorts presented a significantly (P<0.0001) higher number of seropositive pigs. A moderate correlation (r=0.506) was observed between seroprevalence and *Salmonella* isolation from mesenteric lymph nodes, and the VG showed a significant reduction (P<0.0001) in the frequency of *Salmonella* isolation from mesenteric lymph nodes, demonstrating that the vaccination had a protective effect. Veterinary vaccine challenge studies should provide evidence for vaccine efficacy; presenting internal controls and replicating field conditions (Denagamage and others 2007). The authors conducted a clinical trial that followed up seven batches of vaccinated pigs and a similar group of control pigs from birth to slaughter in a multiple-site system, which had a history of high *Salmonella* prevalence. This herd presented a significant reduction in seroprevalence and the number of *Salmonella* lymph node carriers in vaccinated batches at slaughter, which supports studies that proposed the use of vaccines for the control of *Salmonella* infection (Maes and others 2001, Haesenbroek and others 2004, Boyen and others 2008). Live-attenuated vaccines are more suitable for induction of cell-mediated immunity required for an effective protection against facultative intracellular bacteria, such as *Salmonella* species. However, mucosal and serum antibodies against somatic antigens also play a role in host immunity (Haesenbroek and others 2004), and a low level of cross-protection is observed between antibodies induced by different *Salmonella* serovars (Wallis 2001). Therefore, the further
development of a vaccine including an attenuated S Typhimurium strain may be able to induce an even better protection in pigs.

Another issue about the use of vaccines has been the induction of antibodies that interfere with the serological tests adopted by monitoring programs (Leyman and others 2011). In the present study, no seroconversion after vaccination was noticed in the ELISA test biased on seroantigens 1, 4, 5 and 12 of S Typhimurium. It may be related to the fact that somatic antigens of S Choleraesuis (O-6, 7) are not included in the adopted test. However, low frequencies of seropositive pigs have also been reported after the administration of the SC-54 strain vaccine, when tests that included somatic antigens 6 and 7 were used (Maes and others 2001).

In conclusion, in herds with a high prevalence of Salmonella, vaccination with the SC-54 strain can be considered as an additional management tool to reduce the number of carriers in a shorter period of time, even though changes in management and the correction of risk factors remain essential for achieving the target of a low prevalence level in Salmonella control programmes.

References


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