The effect of *Trypanosoma evansi* infection on pig performance and vaccination against classical swine fever

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Abstract

Although *Trypanosoma evansi* is not considered as an important pathogen in pigs, it may interfere with other pathogens or vaccinations by its immunosuppressive nature. In order to determine whether *T. evansi* alters pig performance and induces immunosuppression in pigs, induction of immune responses by vaccination against classical swine fever (CSF) and by immunization with a control antigen, human serum albumin (HSA), was assessed in *T. evansi*-infected and non-infected animals. Although *T. evansi* infection did not have a significant influence on growth performance, feed conversion or PCV, antibody responses against both the test antigen HSA and the CSF vaccine were significantly reduced in *T. evansi*-infected animals as compared to uninfected animals. Moreover, the reduced response against the CSF vaccine appears to be accompanied by a less well-developed protection against CSF with higher fever responses and leukopenia. This immunosuppression might explain the accounts of poor protection of CSF-vaccinated pigs reported in *T. evansi*-endemic areas of Vietnam, and suggests that prior treatments with trypanocidal drugs to improve the efficacy of CSF vaccination, may be justified.

Keywords: Immunosuppression; Classical swine fever; *Trypanosoma evansi*; Pig-protozoa

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1. Introduction

Infections with *Trypanosoma evansi* in pigs are not conceived important and rarely reports of diseased pig herds are received. So far a limited number of studies has been carried out in South-east Asia on *T. evansi* in pigs with various outcomes. Arunasalam et al. (1995) described an outbreak in breeding stock characterized by fever, anorexia, emaciation, abortion and death. Bajyana Songa et al. (1987) noticed also reproductive problems in Thailand while Reid et al. (1999) concluded that the major implication of *T. evansi* infection in pigs laid in their hosting as a reservoir for other livestock species as cattle, horses and buffaloes. Similar as the situation in water-buffaloes, where vaccination failures were described against haemorrhagic septicaemia with a *Pasteurella* vaccine (Holland et al., 2001b), recently a large number of cases of insufficient protection induced by different commercial classical swine fever (CSF) vaccines were reported in Vietnam; vaccinated pigs died with clear symptoms of acute CSF despite being vaccinated. CSF is considered to be one of the most important infectious diseases affecting pigs in many countries in Asia and elsewhere, with numerous outbreaks all year round (Dung and Blacksell, 2000).

Many possibilities for vaccination failures do exist such as vaccine quality, vaccination technique and quality of cold chain. Although vaccination failures due to trypanosome infections have been described in ruminants, so far no investigations were carried out on the possible immunosuppressive role of trypanosome infections in pigs (Whitelaw et al., 1979; Ilemobade et al., 1982; Rurangirwa et al., 1983; Ikeme et al., 1984; Onah et al., 1997, 1998; Holland et al., 2001b).

The main objective of the present study was to investigate if *T. evansi* induces immunosuppression in pigs. To determine whether *T. evansi* induces immunosuppression and may interfere with the development of immunity after vaccination in pigs, *T. evansi*-infected and non-infected pigs were vaccinated against CSF and subsequently challenged with virulent CSF virus. Fever, survival rate, leukocyte and trombocyte counts and specific CSF antibody titres were monitored. All animals received also two immunizations with human serum albumin (HSA) as a control antigen and antibody titres against HSA were monitored. Additionally, we examined the pathological importance of *T. evansi* in pigs. So far several studies were undertaken to investigate the influence of *T. evansi* infection on production in livestock (Payne et al., 1991, 1992, 1993; Pohlpark et al., 1999). However, no such studies were conducted in pigs and to further elaborate on the possible pathological role of *T. evansi* in pigs, daily weight gain, feed conversion and packed cell volume (PCV) of infected and non-infected pigs were monitored after an experimental infection.

2. Materials and methods

2.1. Experimental animals and design

A total of 21 piglets of mixed sex, 5 weeks of age, and confirmed negative for *T. evansi* and CSF virus-specific antibodies (ELISA according to Verloo et al., 2000 and Colijn et al., 1997) were selected. After 3 days acclimatisation, animals were allotted into three equal groups (*n* = 7). The three groups were housed separately and animals were equally...
fed on a restricted diet of commercially available concentrate, the daily amount recorded. Groups A and C were infected with $10^6$ *T. evansi* bloodstream trypamastigotes of the isolate WH ITMAS 101298 (Holland et al., 2001a) while group B was the non-infected control group. Four weeks after infection with *T. evansi*, animals of group A and control group B were vaccinated IM in the right neck, with the commercial CSF vaccine Pestiffa® C strain (Lot no. L73452, Merial, France). Further, all three groups received an IM immunisation in the left neck site with 1 mg HSA (Sigma A-3782). Hence, 1 mg of HSA was dissolved in 0.5 ml saline and subsequently mixed with 0.5 ml Freund’s incomplete adjuvant. Four weeks after the first immunisation, all animals received a booster with HSA. Eight weeks after the CSF vaccination, or 12 weeks after *T. evansi* infection, all three groups were challenged intra-nasally with a virulent dose of a CSF strain isolated from Vietnam (Ky Son district, Nghe An province). Therefore, heparinised whole blood was collected from a viraemic experimentally infected pig.

2.2. Sampling procedures

Plain and heparinised blood samples of all pigs were collected weekly until the CSF virus challenge to confirm the presence or absence of parasites and to determine the PCV and antibodies levels against CSF virus and HSA. *T. evansi*-infected animals were monitored daily for fever, anorexia and survival rate. All animals were weighed every fortnight. After challenge with the CSF virus, blood samples were collected in EDTA at days 0, 2, 4, 7, 10 and 14 post-challenge to determine the number of leukocytes and trombocytes. After challenge, animals were examined daily for body temperature (fever $>$40 °C) and clinical symptoms of CSF, e.g. anorexia, paresis.

2.3. Laboratory procedures

2.3.1. Detection of *T. evansi* infections

To check animals for the presence of *T. evansi*, heparinised blood was analysed with the micro-haematocrit centrifugation technique (MHCT; Van Meirvenne, 1999) and meanwhile the PCV was recorded. Provided the MHCT was negative, heparinised blood (0.25–0.5 ml) was inoculated intra-peritoneally into laboratory bred white mice. The inoculated mice were bled from the tail and a wet blood film was prepared to detect parasitaemia. Mice were checked two–three times a week for 1 month. Antibodies against *T. evansi* were measured by ELISA on *T. evansi* VAT RoTat 1.2 (Verloo et al., 2000) with minor modifications; test sera were diluted 1/400 in PBS-blotto and rabbit anti-pig IgG peroxidase (Sigma A-5670) diluted 1/15000 in PBS–Tween 20 was used as a conjugate.

2.3.2. CSF-specific antibodies

Sera were tested with the Ceditest® CSF virus, a complex-trapping blocking ELISA that uses two monoclonal antibodies directed against the recombinant CSFV E2 antigen (Colijn et al., 1997). ELISA results were expressed as the percentage inhibition ($I(\%) = 100 \times (1 – [OD test serum – mean OD positive reference serum]/[mean OD negative reference serum – mean OD positive reference serum])$ and a cut-off value of 30% was applied: $I(\%) >30\%$ was considered positive, $I(\%) <30\%$ was considered negative. All test sera were
tested undiluted and diluted two-fold in PBS (from 1:2 to 1:8). The titre of a test serum was the inverse of the highest dilution with \( I(\%) \geq 30\% \). In order to detect significant differences between the two groups, the mean titre of each group was calculated and analysed by ANOVA.

2.3.3. HSA-indirect antibody ELISA

The ELISA was previously described by Holland et al. (2001b) and slightly modified. In short, ELISA plates were coated overnight with HSA (Sigma A-3782) diluted in PBS to a concentration of 2 \( \mu \)g/ml and, the next day, blocked and subsequently washed. All test sera collected post-immunisation were diluted in PBS starting at 1/1000 and subsequent two-fold dilutions were prepared until 1/1,024,000. After washing, rabbit anti-pig IgG (whole molecule)–peroxidase (Sigma A-5670) diluted 1/10,000 in PBS was added. As a substrate chromogen ABTS solution was used. The titre of a test serum was the inverse of the dilution having an OD higher or equal to the calculated cut-off. The latter consisted of the mean OD of all pre-immunisation sera (\( n = 21 \)) at dilution 1/1000, plus three times the standard deviation (cut-off = 0.22 OD). In order to detect significant differences between the two groups, the mean titre of each group was calculated and analysed by ANOVA.

2.3.4. Leukocyte and trombocyte counts

The number of leukocytes and trombocytes in EDTA blood samples was determined in a Sysmex® KX21 Coulter counter. Leukopenia was defined as \(< 8 \times 10^6 \text{ cells/ml} \) and trombocyopenia as \(< 200 \times 10^6 \text{ cells/ml} \).

2.3.5. Fluorescence antibody test

To confirm the presence of CSF virus antigen in tissue biopsies from slaughtered animals, the fluorescence antibody test (FAT) was used as described by OIE (1996).

3. Results

3.1. T. evansi screening and infection

All \( T. evansi \)-injected animals (groups A and C) were confirmed parasitologically positive from the first week post-infection onwards, while all animals of the control group remained negative throughout the experiment. From 9 weeks post-infection onwards some infected animals could not be confirmed parasitologically positive anymore. All pigs of groups A and C became serologically positive (\( T. evansi \)-specific antibodies) from day 7 post-infection onwards, while animals of group B remained negative. During the first 12 weeks post-infection, no clinical signs of \( T. evansi \) infection were observed.

3.2. Growth, feed conversion and PCV

No significant differences in daily weight gain and feed conversion between infected and non-infected animals were observed. The 14 pigs infected with \( T. evansi \) (groups A and C)
gained an average of 19.6 kg, while the non-infected pigs (group B) gained 20.4 kg over the same period until challenged with the CSF virus. The infected and non-infected groups had over the 12 weeks period feed conversions of 2.85 and 2.80, respectively.

During the 12-week monitoring period no significant differences in packed cell volume between the groups could be observed: the mean PCV levels remained stable around 35, with no major fluctuations in either group.

3.3. Clinical symptoms after CSF virus challenge

One animal of the B group succumbed after chronic diarrhoea (Clostridia spp.) on the day of challenge. After the CSF virus challenge, three out of seven animals in the non-vaccinated T. evansi-infected group C were found recumbent and were subsequently euthanised on days 2, 7 and 8, respectively. The FAT on spleen samples of these three pigs were positive for CSF. The other four animals in this group lost their appetite and were dull for a number of days. In the vaccinated T. evansi-infected group A, four animals had reduced appetite for 2–5 days after challenge. In the vaccinated uninfected group B, however, no symptoms were observed.

Body temperature was recorded daily for 14 days after CSF challenge. The percentage of recordings of animals with fever (>40 °C) over this 14-day period was 22, 11 and 42 for groups A, B and C, respectively. The daily percentages of animals with fever are represented in Fig. 1. Upon CSF challenge, more animals with fever were detected in the T. evansi-infected groups A and C than in the non-infected group B, and could be detected for a longer time. For the CSF-vaccinated groups, more animals with fever were detected in the T. evansi-infected group A than in the non-infected group B and also for a longer time (Fig. 1).

![Fig. 1. Percentage (%) of animals per day with fever after CSFV challenge. T. evansi-infected and CSF-vaccinated (group A (○)), only CSF vaccinated (group B (□)) and only T. evansi-infected animals (group C (▲)).](image-url)
3.4. Leukocyte, thrombocyte counts

In group A, no leukopenia could be detected throughout the 14 day monitoring period after the CSF virus challenge. One animal of group B had leukopenia on day 4 and recovered on day 7. In group C, three animals were found with leukopenia; one on day 4 and one on day 7, both succumbing the same day, while the third animal was found with leukopenia on day 10 but fully recovered on day 14.

Thrombocytopenia was found in group A in two animals on days 2 and 4, one animal only on day 2 and one animal only on day 4. In group B, low thrombocyte levels were found, in one animal on days 2 and 4, one animal on days 4 and 7 and one animal only on day 2. In group C, low thrombocytopenia levels were found in two animals on days 2 and 4 of which one died, one animal on days 2, 4 and 7 which subsequently succumbed and, finally one animal on days 4 and 7 which also died.

3.5. CSF-specific antibodies

All pigs vaccinated with the CSF vaccine (groups A and B) sero-converted as measured by the Ceditest® CSFV. CSF-specific antibody titres increased gradually from day 14 onwards to maximum levels at day 35. On day 28, 35 and 42 post-vaccination, titres were significantly higher in the non-infected group B than the infected group A (Fig. 2). Animals of the non-vaccinated control group remained sero-negative until the day of CSF-challenge.

3.6. HSA-indirect antibody ELISA

Upon HSA-immunization, all 21 pigs sero-converted against HSA as measured by ELISA. After primary immunisation the mean titres of the T. evansi-infected groups A and C and the uninfected group B increased gradually in both groups with a non-significantly higher
4. Discussion

The experimental infections of pigs with the *T. evansi* buffalo strain WH ITMAS 101298 did not induce major clinical symptoms: growth performance, feed conversion and PCV levels were not affected significantly compared to the uninfected controls. The absence of clinical symptoms or only low influence on growth, feeding or PCV levels confirms findings by others (Dr. Phan Dich Lan, Vietnam, personal communication, Reid et al., 1999) that *T. evansi* experimental infection in (young) pigs has a limited pathology. Other studies, however, investigating the influence of natural *T. evansi* infection on pig production, demonstrated fertility problems (Arunasalam et al., 1995; Bajyana Songa et al., 1987). It should, however, be noted that all experimental infections including the present study, were carried out on young animals with non-pig isolates.

The challenge with the CSF virus was lethal for three out of seven non-vaccinated pigs while the remaining four animals had fever for at least three days post-challenge. Also the percentage of recordings of thrombocytopenia in the non-vaccinated group was nearly twice (32%) those of the vaccinated groups A and B (17%). Conversely, leukopenia appeared not to be of major importance for the challenged non-vaccinated group. On the contrary, all the vaccinated animals survived the virus challenge confirming the protective value of the vaccine that prevents mortality and major clinical symptoms. However, some animals in the *T. evansi*-infected group A showed loss of appetite for 2–5 days post challenge.

The present study demonstrates that the presence of a *T. evansi* infection lowers the immunoresponsiveness of fattening pigs to concurrent immunisations. Indeed antibody
responses against both the test antigen HSA and the CSF vaccine were significantly reduced in *T. evansi*-infected animals as compared to uninfected animals. This reduced response against the CSF vaccine appears to be accompanied with a less well-developed protection against CSF. A more profound loss of appetite post-challenge and twice the number of days with fever were observed in the *T. evansi*-infected group as compared to the non-infected group. However, no differences in leukopenia, trombocytopenia or survival rates were observed between the groups.

These data support the findings in ruminants on the immunosuppressive nature of trypanosome infections (Whitelaw et al., 1979; Ilemobade et al., 1982; Rurangirwa et al., 1983; Ikeme et al., 1984; Onah et al., 1998; Holland et al., 2001b). In mice and cattle (Sileghem et al., 1991; Sileghem and Flyn, 1992), it has been demonstrated that *Trypanosoma* infections reduce T cell responsiveness. In both the species, T cell proliferation was profoundly suppressed upon mitogenic stimulation and mediated by macrophage-like suppressor cells, leading to suppressed interleukin-2 secretion and impaired expression of the interleukin-2 receptor. A possible explanation for the reduced antibody responses in our infected pigs might thus reside in a T cell suppression induced by the concurrent *T. evansi* infection. Indeed, the induction of a good antibody response needs a good T helper cell response as the latter provides help to the B cell though delivery of cytokines.

In conclusion, our results suggest that the inductive capacity to mount immune responses on heterologous antigens may be suppressed in *T. evansi*-infected fattening pigs and consequently that *T. evansi* infection might interfere with the development of protective immunity upon heterologous vaccinations. This immunosuppression may explain the accounts of poor protection of CSF-vaccinated pigs reported in *T. evansi*-endemic areas of Vietnam. Therefore, it is suggested that in *T. evansi*-endemic areas a treatment with trypanocidal drugs may enhance the efficacy of CSF vaccination.

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References


