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Commentary

A live vaccine against *Neospora caninum* abortions in cattle

Dear Editor

We think there is an opportunity to debate the topic of "*Why the cattle industries and associated veterinary pharmaceutical industry, veterinarians and farmers alike should not be afraid of using a live vaccine against Neospora caninum-associated abortion loss".*

N. caninum has emerged as a major cause of abortion in dairy and beef cattle and it is estimated to be responsible for losses in excess of a billion dollars annually, in cattle industries worldwide [1]. Yet, after more than 25 years of research on this parasite, the control options for this disease appear to be reduced to interrupting the life cycle of this parasite. A commercial, inactivated vaccine for the prevention of bovine neosporosis was introduced to markets more than a decade ago and subsequently withdrawn from sales, presumably due to its very low efficacy [2], as observed with many other parasitic diseases [3].

The remaining options that are proposed include reproductive management, (selective culling through the use of diagnostic tools), limiting access of canids (the known definitive hosts: dog, wolf, coyote and dingo [4, 5]) and their fecal material to water and food supplies of cattle to the proper disposal of tissues produced by abortions. The sylvatic life cycle of this protozoan involves rodents, birds and many other mammals and is difficult to control [6]. An efficacious, live vaccine would seem to offer a simple and attractive alternative to these above options [7]. Fortunately, neosporosis does not appear to be a zoonotic disease [8].

N. caninum has such an intriguing adaptation to cattle as the intermediate host that vertical transmission occurs efficiently without any clinical sign, but for the occasional abortion and, sporadically, neurological clinical signs in newborn calves. Stress, genetics

(dairy breeds appearing more susceptible to infection with *N. caninum* than beef breeds [1]), immunosuppression (mycotoxins [9], Bovine Viral Diarrhoea virus [10]), management have all been suggested as risk factors for the expression of disease. In addition, postnatal transmission occurs, involving canids as the definitive host, allowing the parasite to complete its life cycle [11].

Costly management control options include the culling of infected animals. Hitherto no drug has been marketed to prevent either abortions or infections in cattle [6, 11], although experimentally a tachyzoite challenge could be curtailed with frequent doses of toltrazuril [12]. As the disease causes serious reproductive losses, one would expect that generous funding from the public and private sector would be available for the development of tools for the control of the disease.

Many research groups previously working on the related protozoan *Toxoplasma gondii* drew from their expertise, methodologies and techniques to understand this economically important coccidian parasite. Continuing efforts have demonstrated that the only way to prevent abortions appears to be through exposure to the parasite before pregnancy, just as is the case with toxoplasmosis in humans and sheep [13, 14]. For many livestock diseases, live vaccines are used as effective tools for the prevention of serious disease, such as toxoplasmosis, theileriosis, babesiosis and coccidiosis [7]. Moreover, naturally attenuated strains have proven to be useful in preventing abortions in neosporosis [15, 16]. Yet, a live *Neospora* vaccine has not reached the market, despite the fact, that these “proof-of-concept” trials have demonstrated high efficacy against an exogenous (*de-novo* infection of the pregnant dam, which crosses the placenta) transplacental infection. Additional work is needed to assess the ability of such “vaccines” to prevent endogenous (recrudescence of an already established, chronic infection of the dam during pregnancy) transplacental infection; this has not yet been assessed.

Infection with *N. caninum* during pregnancy can have different outcomes, often depending on the time point of infection and the immune status of the fetus. If fetal infection ("not cow infection") occurs in the first trimester of gestation, the outcome is often abortion; transplacental transmission of the parasite is expected to be low in this time of pregnancy . However, calves born from *Neospora*-positive dams might continue to develop through gestation, and are either born weak, show neurological signs of disease later in development, or can also be completely unaffected [17]. In the latter case, such calves can then, in turn, give birth to *N. caninum* infected offspring, which are either affected by the disease or not. This complicates the situation immensely, and renders the development of a vaccine, no matter whether a live or a subunit version, a difficult task. A vaccine that can both, prevent abortion as well vertical transmission, might be difficult to achieve [18] and be seen by the industry as being too technically challenging.

Another important factor that makes vaccine development in neosporosis difficult is the fact that there is no small animal laboratory model available that reliably simulates the placental environment in cattle. Ultimately, safety and efficacy need to be shown in the target species, i.e. primarily cattle. Therefore, “proof-of-concept” studies in the rodent model, although possibly an important staging point in the decision making process of the pharmaceutical industry, have to be treated with caution, and do not necessarily predict the outcomes in cattle [18], and their utility might thus be queried even on animal welfare grounds. Small ruminants, in particular sheep may provide a further model system [19].

Disadvantages that might be seen to be associated with live vaccines include the potential for reversion to pathogenicity, costly production and distribution channels, and latency in the intermediate host [7], which might also explain why no live vaccines have been commercially produced for neosporosis yet. If cattle vaccinated with a live, attenuated vaccine were to suffer abortions, this would potentially expose the vaccine manufacturer to

lawsuits. Live vaccine production would likely rely on cell-culture-derived organisms the production process of which is labour intensive in its maintenance. Live vaccine production also present challenges in terms of parasite preservation and viability thereafter, although the successful production and distribution of Toxovax® (a live vaccine for the prevention of *T. gondii* abortions in sheep) shows this is achievable through a "made to order" process. As live vaccines might possibly be expected to result in chronic infection of the host, a residual risk exists that the life cycle could ultimately be completed again, if tissues from vaccinates was to be fed to canid definitive hosts. With Toxovax®, the tachyzoites of *T. gondii* appear to have lost the ability to encyst (and thus persist) in the vaccinated host; a live vaccine for *N. caninum* ideally would use similarly modified life stages of that parasite. Also, cattle that are vaccinated with a live isolate might be difficult to distinguish from their naturally *N. caninum* infected cohorts and a diagnostic approach that facilitates DIVA (**D**ifferentiating **I**nfected from **V**accinated **A**nimals) may be required.

Molecular techniques are, however, available to distinguish *N. caninum* vaccinated from naturally infected animals, including the possibility for genetic characterization of isolates [20, 21]. These techniques also might give rise to the possibility of the development of live marker vaccines with gene deletions for specific proteins, which could, in turn, be used in a given serological test to distinguish those cattle thus vaccinated from those animals that were naturally infected.

Currently, no vaccine composed of recombinant antigens is commercially available for diseases caused by apicomplexan parasite. However, some vaccines based on native antigens are available. CoxAbic™ is composed of affinity-purified gametocyte antigens from *Eimeria maxima* and confers protection to hens and their offspring against coccidiosis by transmission of specific antibodies via egg yolk [22]. Another marketed vaccine composed of soluble antigens from two *Babesia* species (Nobivac Piro™) confers protection against babesiosis in

dogs [23]. The success of these vaccines in preventing parasitic disease provides "proof-of-concept" that subunit vaccines can be produced. They are also considerably cheaper to manufacture than live vaccines and this may influence the decision-making process of the leaders of pharmaceutical companies, deciding that focus on the development of recombinant subunit vaccines is worth the trouble.

While recombinant antigens may be more attractive from a financial point of view, they have, in the case of neosporosis, not yet shown similar efficacy [18]. There is also a large body of scientific evidence for apicomplexan parasites which shows the live vaccination route is still the most promising [7]. While, however, these recombinant and sub-unit approaches are being actively investigated, and further work should investigate these, the animal health and primary industry, and veterinarians should nevertheless, in the meantime, invest in the development of a live vaccine against bovine neosporosis. This seems to be an approach that could lead to an efficacious vaccine for the prevention of abortions in cattle, and should be investigated more thoroughly.

Refinement of the current experimental vaccine regimes might yield vaccine candidates that are cheaper to produce: reduced doses still sufficient to induce protective immunity should be investigated, as they might also decrease the cost of production. Vaccination before puberty may be another option. Application of the vaccine onto mucosal surfaces could be an alternative as well, making for easier and cheaper application on the farm.

Recent publications have calculated the considerable losses inflicted by *N. caninum* on cattle industries world-wide [1]. Vaccination, at least at this stage, appears to be the only viable intervention strategy that appears feasible. The development of a live vaccine against neosporosis should be pursued, in order that its benefits can be transferred as soon as possible

to veterinarians and farmers in order to prevent the reproductive, productive and economic losses caused by *N. caninum*.

Yours sincerely

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Conflict of interest statement

The authors have no known conflicts of interest

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