A live vaccine is safe and efficient to protect poultry against histomonosis

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Abstract

The protozoan parasite *Histomonas meleagridis* is the aetiological agent of histomonosis, a highly fatal disease in turkeys with less mortality in chickens. Following the ban of licensed drugs to be used for prophylactic and therapeutic treatment the number of outbreaks increased in recent years, without any suitable and legal option to combat the disease. In various cases the whole flock had to be killed in order to reduce suffering of animals highlighting the importance histomonosis has gained also in the light of animal welfare. Considering the difficulties for licensing new drugs to be used in food producing animals, vaccination would be a new option to combat the disease. In the following presentation the efforts are consecutively presented leading to a safe and efficient vaccine, without any side effects on health and performance of birds. This vaccine could be the basis to prevent suffering and loss of animals due to a non-treatable disease.

Keywords: *Histomonas meleagridis*; histomonosis; live vaccine; safety; efficacy

1. Introduction

Histomonosis (syn. histomoniasis or blackhead disease) is a parasitic disease caused by the flagellated protozoan *Histomonas meleagridis* [1]. It is most harmful in turkeys with up to 100% mortality and a lower mortality in chickens [2]. Due to the actual drug legislation for food producing animals, prophylactic or therapeutic intervention is no longer possible within the EU and very much limited in the USA [3, 4, 5]. Consequently, numerous outbreaks occurred in recent years since the complete ban was

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implemented in 2003. Searching for new drugs is hampered by possible side effects they may cause as demonstrated for the aminoglycoside antibiotic paromomycin [6]. According to this report of the European Food and Safety Authority, the drug selects for resistance and cross-resistance against other aminoglycosides among bacteria present in the gut, raising serious concerns. Because prophylaxis and therapy was performed over decades with chemical substances, vaccination would be a completely new approach to combat the disease.

The lack of knowledge about the protozoan parasite *H. meleagridis* is a certain hindrance for vaccine development. Earlier studies had shown that the protozoan might survive only for a short time under adverse conditions [7]. Even after completion of a successful development this may limit the efficient application by mass vaccination technology like spray or drinking water, commonly used in poultry. Efficacy and safety of the vaccine without any negative influence on the general constitution of the bird are the most important parameters to be investigated prior to release of such a product.

2. Materials and Methods

Different animal trials were performed to address the likelihood of vaccination against histomonosis. All trials were accompanied with newly developed laboratory methods suitable to determine the presence of the *H. meleagridis* in tissues and excreted faeces. Recently established ELISA [8] was applied to confirm the infection and spread of the vaccine or challenge to in-contact birds. All trials were discussed and approved by the institutional ethics committee and licensed by Austrian law. The detailed description of every experiment can be found in the publications listed in the references.

3. Results and Discussion

The first step towards vaccine development was the establishment of a defined culture capable to be stored and preserved accordingly. Growing of numerous protozoa is influenced by certain factors and very often a complicated and cumbersome procedure [9]. To obtain a well defined culture of *H. meleagridis* micromanipulation was adopted, as this technology enables the selection of single cells [10]. For correct identification a nomenclature was introduced stating the type of protozoon/species of bird/country of origin/diagnostic number-clone number/year of isolation (Hm/Turkey/Austria/2922-C6/04). A starter medium including defined bacteria was needed for optimal growth condition. Such xenic cultures can be frozen down in the presence of DMSO as cryoprotectant, enabling long term storage. In a first step such a culture was found highly virulent after a few passages, inducing 100% mortality and spread to in-contact birds [11].

Based on the known experience by attenuating other micro-organisms through long term passaging in *vitro*, aliquots of such a culture were passaged every 3-4 days. In the past several authors have reported divergent results performing such kind of investigations. As a consequence of this and based on own experiments Lund *et al.* [12] concluded that attenuation is not possible by performing in *vitro* passages. Instead such an outcome is the result of a selection process occurring in a culture which contains flagellates of different virulence. Based on our results this hypothesis could be contradicted by applying clonal cultures traced back to a single cell. Following 95 passages the parasites were already attenuated with no mortality in experimentally infected turkeys [13]. This process was even pronounced continuing *in vitro* cultivation for up to 295 passages. In the same study it could be shown that all birds which were previously infected with attenuated protozoa were completely protected against a severe challenge 4 weeks post vaccination with virulent histomonads. Not only the vaccine, the virulent parasites as well
spread efficiently to in-contact birds. In comparison to the live parasites, killed histomonads injected intramuscularly were found non-protective. This is in agreement with an earlier study in which serum of surviving birds transmitted to susceptible animals was unable to protect these birds [14]. This was later confirmed by another group comparing active and passive immunization [15].

Even though successful protection could be demonstrated in the initial study the application of the vaccine via the cloaca of the birds reflected a certain hindrance for mass application under field conditions. Similar to the results mentioned above Hu and McDougald [16] demonstrated that histomonads could be transmitted from infected to in-contact birds in the absence of any vector. A phenomenon described as cloacal drinking was given as possible explanation for the uptake of fragile parasites into the caeca. Performing constant re-isolation of histomonads from faeces we were able to demonstrate the successful spread of the flagellates already 2 days post infection, arguing for another route of transmission. Considering birds behaviour of constant picking at faeces the oral uptake of parasites seem to be the first choice. Consequently, oral infection was favoured in a separate study using virulent histomonads. The effective oral infection could be demonstrated in several experimental settings and the parasites spread again to in-contact birds. Birds were deprived from food and water for 5h following infection in order to increase efficiency of infection. The ability of *H. meleagridis* to induce cyst-like stages under *in vitro* conditions may contribute to the success of oral infection as these stages are more suitable to survive the harsh conditions within the digestive tract [17].

As a consequence of the above mentioned study it was obvious to investigate in a follow up experiment whether attenuated parasites can be used for oral vaccination. For this approach day-old turkeys were vaccinated in order to induce an early protection [18]. Birds kept in different groups were challenged either at 2 or 4 weeks post vaccination. Challenging of vaccinated birds at 2 weeks post vaccination induced partial protection as some birds died, whereas all birds which were challenged 4 weeks post vaccination survived indicating complete protection. In a separate group birds were only vaccinated omitting any challenge. Weekly blood samples were taken to confirm infection which was noticed three weeks post vaccination based on presence of IgG. The antibodies stayed above the cut-off value until the study was terminated at 16 weeks post vaccination indicating the potential of the developed ELISA. In addition, non adverse effect on body weight gain could be noticed in these birds.

In conclusion, a consecutive series of experiments was presented outlining the development of a live vaccine to protect turkeys from fatal histomonosis. Beside its efficacy the vaccine is safe and has no negative effect on performance. Therefore, for the first time it could be demonstrated that live vaccination based on clonal cultures is a new and suitable concept to prevent histomonosis in poultry avoiding any residues in meat products obtained from such birds.
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References


