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Monitoring the uptake of live avian vaccines by their detection in feathers

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ABSTRACT

Protection against diseases caused by the avian viruses, Marek's disease, Infectious laryngotracheitis, chicken anemia and turkey meningoencephalitis is achieved by live vaccines. The application quality is important to assure proper uptake in commercial flocks. We describe a novel evaluation method for the vaccination process by sequential monitoring the vaccine viruses in feathers. Feather collection is easy, non-invasive and non-lethal for the birds, therefore advantageous for monitoring purposes. To demonstrate the vaccine virus presence, an innovative assay of nested real-time amplification was approached because vaccine viruses presence *in vivo* is less abundant comparing to virulent wild-type isolates.

The Marek's disease virus vaccine virus, Rispens/CVI988, in feathers of commercial flock was detected from 4 to 7 days and for at least 3 months post-vaccination, until the survey stopped. As the drinking water route was newly adopted for Infectious laryngotracheitis vaccination, one or two vaccine doses/bird were administered. The virus uptake was detected in feathers between 2 and 20 days-post-vaccination. With a doubled vaccine dose the positivity bird rate was higher. For the first time the chicken anemia vaccine virus presence in chicken feathers was demonstrated between 14 and 35 days-post-vaccination. No previous studies were available, thus in parallel to feathers the vaccine virus was demonstrated in the livers and spleens. The turkey meningoencephalitis vaccine virus uptake in turkey feather-pulps is even more innovative because this is the first turkey virus amplified from feather-pulps. The vaccine virus presence resemble the kinetics of the other 3 viruses, 3–21 days-post-vaccination. Detecting the specific antibodies following vaccination possessed a lower sensitivity than vaccine virus demonstration in feathers. In summary, the presented assay can be adopted for the quality evaluation of the vaccination process in poultry.

1. Introduction

Vaccination of commercial poultry is crucial for the industry profitability. Vaccine administration is diverse, including individual bird injections, manually or automatically, or mass-vaccination through drinking water, spray or *in ovo* vaccination. In addition to the vaccine efficacy to protect against virulent pathogens, its application is essential. However, that aspect attracted less attention. Typically, vaccination application is monitored by the antibody levels following vaccination, however, that approach is indirect and not universally applicable for viruses that elicit mainly cell-associated immune responses.

No direct assays for vaccine application evaluation exists, therefore we suggest an innovative assay by spotting the spread of live vaccine viruses systemically, by their detection in feather shafts of vaccinated birds. The feathers are advantageous for monitoring purposes as their collection is easy and not lethal. Avian viruses replicate in epithelial cells of the feather follicles, in lymphocytes and myelocytes circulating and reaching the feather calamus cavity and accumulate at that site, forming the feather pulps, which then represent the systemic blood.

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The present study focuses on four avian vaccine viruses, Marek's disease (MDV) [1], chicken anemia (CAV) [2], Infectious Laryngotracheitis (ILTV) [3] and the turkey flavivirus, Turkey Meningoencephalitis Virus (TMEV) [4].

MDV presence in the feather follicle epithelium (FFE) was explored extensively, revealing the infective virus productive replication and spread by dust and dander [5–8]. MDV causes tumors, immunosuppression and neurological symptoms. MDV vaccine efficacy and uptake was studied simultaneously in experimentally-infected and commercial vaccinated layers [9–11]. The present study anchored on MDV and launches the concept of evaluating the vaccine application of additional poultry vaccines.

CAV infections have serious economic impact with evident disease or sub-clinical infection and immunosuppression, reflecting negatively on diseases caused by other pathogens, and affecting vaccination efficacy [2]. CAV detection in feather tips was firstly reported in experimental infection trials, and then applied for diagnosis [12–15]. For MDV and CAV no system of direct vaccine application evaluation exists today.

The herpesvirus ILTV causes severe respiratory disorder and latent infections with concurrent reactivations due to stressful physiological conditions through lifetime [3]. ILTV live vaccines are administered via eye-drop, spray or drinking water. Alternatively, the cloacal vent-brush application has been successfully practiced in Israel [16] until recently when the drinking water route was introduced for the ILTV vaccine. A subjective, indirect method of evaluating "takes" is used to monitor the ILTV vaccine application by vent-brush [16]. Our pilot study introduced the direct method for ILTV vaccine uptake [17] showing the feather shafts as a site of vaccine virus presence. In this study that approach is further substantiated and expanded.

The turkey flavivirus TMEV causes a neuroparalytic disease that is controlled by vaccination with a live attenuated virus [18]. We recently reported on TMEV detection in feather-pulps and on the DIVA assay development (Differentiating Infected from Vaccinated Animals) [19] by which the TMEV vaccine application was explored. The TMEV vaccine application is indirectly evaluated by the Haemagglutination Inhibition (HI) antibody titer [26].

The accuracy and applicability of findings obtained from experimental infection trials to commercial flocks cannot be extrapolated. For that reason we now aimed to reflect "real-life" situations and to provide novel operative tools for evaluating commercial vaccination application by focusing on commercial flocks which are vaccinated by commercial teams.

2. Materials and methods

2.1. Vaccines

The MDV vaccine was cell-associated bivalent serotype 1 + 3, MDV-1/Rispens/CVI988+HVT, (Merial, Ltd. and Zoetis, Ltd.).

The CAV vaccine was AviPro-Thymovac, strain Cux-1, LAH, Ltd.

The ILTV vaccine was Vir101/Biovac, Ltd.

The TMEV vaccine, was strain JQ4E4 [16], Phibro, Israel.

2.2. Commercial flocks and feather sampling

Table 1 detail the commercial flocks, vaccination route, vaccination age, days post-vaccination (dpv), and total birds sampled. The chickens were ROSS 308, Aviagen, U.K. and the turkeys were BUT, Aviagen, UK. All flocks were healthy and all flocks at each farm were uni-age, meaning that all birds received the vaccination at once. The chicken flocks were housed in windowless poultry houses, and the turkey flocks were housed in isolated open sheds.

To increase flock coverage, 3–4 feathers were sampled from individual birds and pooled as follows:

MDV vaccination: 4 pools were created from feathers of 20 chicks, covering 580 birds.
CAV vaccination: 5 pools were created from feathers of 15 birds, covering 360 birds.
ILTV vaccination: 5 pools were created from feathers of 25 birds, covering 685 birds.
TMEV vaccination: 2 pools were created from feathers of 10 turkeys, covering 300 birds.

To increase the flock coverage different birds were sampled at each sampling time. As shown in Table 1 two commercial flocks were studied after their MDV and CAV vaccination. Each flock was vaccinated at 1 day-old against MDV and several months later for CAV, therefore the same flock can be surveyed for both vaccine viruses post-vaccination. Accordingly, flock No. 2, detailed for the MDV vaccine, has been surveyed also after its vaccination with the CAV vaccine, and was denoted flock No. 1 regarding the CAV vaccine. As the CAV vaccination was performed at 67 days of age, all the feather DNA samples, that were obtained before the CAV vaccination were tested also for CAV, as reference of the background exposure to CAV.

2.3. Types of feathers sampled

Two types of feathers were used, mature feathers, with or without bloody pulp and immature feathers containing vascular pulp. MDV and CAV replicate in the FFE, are excreted to feather cavities, and deposit on feather walls [12,20]. Extensive analyses of mature and immature feathers of MDV- and CAV-infected chickens revealed similar amplification abilities in both feather types, therefore both types were used. For ILTV and TMEV detection only immature pulp-containing feathers were used, reflecting systemic viremia.

2.4. Nucleic acid purification from feathers

For MDV and CAV detection feather tips were cut in small slices and DNA was purified using Maxwell 16-Tissue DNA kit (Cat. #1030), Promega, Madison, WI, U.S.A., according to the manufacturer's instruction. For ILTV and TMEV detection feather-pulps of 3–4 feathers were squeezed and adjusted to 50% (w/v) with Phosphate-buffered Saline (PBS). The homogenate was incubated on ice (45 min) vortexed repeatedly, and centrifuged (3000*g*-10 min). RNA was extracted using the QIAamp®-Viral RNA Kit (QIAGEN, Ltd., Valencia, CA. USA) according to the manufacturer's instructions.

2.5. Nucleic acid purification from trachea exudates and from visceral organs

DNA was purified from spleen, livers and trachea exudates using Maxwell 16 Tissue DNA Cat. # 1030 kit, Promega, Mad. WI, U.S.A., according to the manufacturer instruction. Small pieces of the internal sections of frozen organs, to avoid external contamination, were excised with scalpels and applied to the DNA extraction kit, as previously described [14]. To obtain trachea exudates, the trachea was cut to open its inner face and then the internal content was scrapped with a scalpel and applied to the DNA extraction kit.

2.6. Amplification

Primers and probes for all amplification systems are described in Table 1 (Supplement). Feather tip DNAs were pre-amplified by

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