Assessment of impact of a novel infectious bursal disease (IBD) vaccination programme in breeders on IBD humoral antibody levels through the laying period

Daniel Parker,1,2 Sjaak de Wit3

ABSTRACT

Objectives: The purpose of this study was to assess whether broiler breeders vaccinated in ovo with a Vaxxitek (HVT&IBD) (Merial) plus an inactivated IBD vaccine prior to the onset of lay had a significantly different humoral IBD antibody response to broiler breeders vaccinated solely with Vaxxitek (HVT&IBD) (Merial) in ovo. In addition, maternally derived antibody (MDA) passed to the progeny of these two breeder flocks was also compared at three time points during lay.

Design: The study was a case–control study where the two flocks were the same breed, reared on the same farm and transferred to the same laying farm, the only difference between the flocks being the IBD vaccination programme. The humoral IBD antibody response in the two breeder flocks and their progeny was measured using two commercial ELISA tests and a serum neutralisation (SN) test.

Results: There was no significant difference (P<0.05) in the humoral IBD antibody response in the two breeder flocks as measured by ELISA test except at a single time point at 22 weeks when measured by BioChek ELISA and at 60 weeks when measured by IDEXX ELISA. There was no significant difference (P<0.05) in the humoral IBD antibody response in the two breeder flocks as measured by SN test except at 27 and 55 weeks. There was no significant difference (P<0.05) in the levels of MDA passed to progeny of these two breeder flocks as measured by ELISA or SN test.

Conclusions: It is proposed that a single vaccination with Vaxxitek (HVT&IBD) (Merial) will provide a similar level of IBD protection to breeders and their progeny as a Vaxxitek (HVT&IBD) (Merial) plus inactivated IBD vaccine, vaccination programme. This novel IBD vaccine provides additional options for vaccination programmes for broiler breeders without impacting on the protection of the broiler progeny.

Infectious bursal disease (IBD) is an economically important disease in poultry worldwide (Van Den Berg 2000). The disease in broilers is characterised by mortality, immunosuppression due to destruction of B cells and macrophages and decreased economic performance (Sharma and others 2000, Van Den Berg 2000). In broilers, current strategies for prevention and control of IBD include passive immunological protection, hygiene and vaccination on the broiler farm with live attenuated vaccines (Fussell 1998, Flensburg and others 2002). Early passive protection of the broiler against infection can be conferred through maternally derived antibody (MDA) (Naqi and others 1983, Lutticken 1997). For this, it is common to use inactivated IBD vaccines in breeders that have been primed earlier with live IBD vaccine in the rearing period to generate levels of MDA in progeny chicks. This elevated MDA will confer early protection against IBD infection in these progeny (Eidson and others 1980, Wood and others 1981). Vaxxitek HVT+IBD (Merial Animal Health Ltd) is a novel vector vaccine in which the IBD virus gene (VP2 gene) coding for VP2 protein of IBD virus has been inserted into a turkey herpes virus (Bublot and others 2007). This vaccine when inoculated into chick causes the expression of the IBD VP2 protein with subsequent antigen/antibody response by the birds (Darteil and others 1995). Antibodies to the IBD VP-2 protein are recognised as being the most important of the neutralising antibodies that confer protection against infection (Fahey and others 1989). Protective levels of MDA to IBD have been demonstrated in progeny of Vaxxitek HVT+IBD vaccinated breeders (Lemiere and others 2013). Knowledge of the antibody development during the laying period is important as it has direct consequences for the age that the progeny can be vaccinated using conventional live IBD vaccines (Block and others 2007). The purpose of this study was to compare (under British field
conditions) the humoral IBD immune response in breeders vaccinated with a single dose of Vaxxitek HVT+IBD in ovo at 18 days of incubation with breeders vaccinated with Vaxxitek HVT+IBD in ovo at 18 days incubation plus an inactivated IBD vaccine at transfer to the laying farm (18 weeks). Humoral antibody response in the breeders and the transfer to the progeny was measured by two commercially available ELISAs and virus neutralisation tests at various time points through the laying cycle.

**METHODS**

**Breeders**

Cobb 500 breeders were vaccinated in ovo at 18 days incubation in the parent hatchery with Vaxxitek HVT+IBD and Cryomarex Rispens (CVI 988) (Merial Animal Health Ltd) and placed on the same rearing site. All birds received the same vaccination programme in rear, no other live IBD priming vaccine was administered in rear or in lay. Prior to transfer at 18 weeks, multivalent oil emulsion vaccine Nobilis RT+IBmulti+G+ND (MSD Animal Health Ltd) containing an inactivated IBD antigen (D78 strain) was administered to 50 per cent of the birds (inactivated IBD group). A multivalent oil emulsion vaccine Gallimune 407 ND+IB+EDS+ART (Merial Animal Health Ltd) without an IBD antigen was administered to the remaining 50 per cent of the birds (Vector IBD group). Both treatment flocks were housed on the same laying farm with two houses allocated to each treatment. Clotted blood samples were collected from the breeders when they were 15 weeks old, prior to the administration of the inactivated vaccine. Further clotted blood samples were collected from both groups when the birds were aged 22, 27, 32, 36, 41, 45, 56 and 60 weeks old on the laying farm. Serum was harvested from the clotted blood samples and assayed for IBD antibodies levels using two commercially available ELISA tests and a virus neutralization test.

**Progeny**

At three time points in the laying cycle, hatching eggs from the two treatment groups were set separately for incubation. On hatching, day-old chicks were blood sampled to assess levels of maternally derived IBD antibodies using two commercially available ELISA tests and a virus neutralisation assay.

**ELISAs**

Two commercially available ELISAs (BioChek CV, Gouda, The Netherlands), IDEXX FlockChek standard (IDEXX Corporation, Westbrook, Maine, USA), for the detection of antibodies directed against IBD were assayed as indicated by the manufacturer’s instructions.

**Virus neutralisation assay**

The assay was based on the method described by Skeeles and others (1978) and Lutticken and Cornelissen (1981) with some modifications. The end point of the virus neutralisation titre (VNT) on a serum sample was determined to be the reciprocal of the highest dilution, expressed in log2, which completely neutralised 100 TCID50 of IBDV D78 strain.

**Statistics**

Analysis of variance (ANOVA) and Tukey test were used to compare the difference in the mean IBD titres of the vector and inactivated IBD groups at the various ages through the trial. ANOVA and Student t test were used to compare the difference in mean titres between vector and inactivated IBD groups at each sampling time for each of the three serology test assays.

**RESULTS**

The mean IBD antibody levels in the breeder flocks at the nine time points in the rearing and laying cycle as measured by VNT and ELISA are tabulated in Tables 1–3. The mean IBD antibody levels from the day-old progeny of the breeder flocks at the three time points in laying cycle as measured by virus neutralisation test and ELISA are tabulated in Tables 4–6.

The geometric mean titre (GMT) in the breeder birds during the laying period were compared with GMT titres of the progeny at day of hatch. The percentage of antibody transfer was determined and is listed in Table 7.

| Age  | No. | Mean       | 95% CI     | No. | Mean       | 95% CI     |
|------|-----|------------|------------|-----|------------|------------|
| 15   | 10  | 16.1a      | 15.3 to 16.9| 10  | 16.0a      | 15.4 to 16.6|
| 22   | 10  | 17.0a      | 16.2 to 17.8| 10  | 17.0a      | 16.4 to 17.6|
| 27   | 18  | 16.9a      | 16.3 to 17.6| 20  | 16.4b      | 15.9 to 16.8|
| 32   | 20  | 15.9a      | 15.3 to 16.5| 20  | 16.1a      | 15.6 to 16.5|
| 36   | 20  | 15.6a      | 15.0 to 16.1| 19  | 16.1a      | 15.7 to 16.5|
| 41   | 18  | 16.6a      | 16.0 to 17.2| 20  | 16.6a      | 16.2 to 17.0|
| 45   | 18  | 16.8a      | 16.2 to 17.4| 19  | 16.8a      | 16.3 to 17.2|
| 56   | 20  | 17.0a      | 16.4 to 17.6| 19  | 16.6b      | 16.2 to 17.1|
| 60   | 19  | 16.7a      | 16.1 to 17.3| 20  | 16.7a      | 16.3 to 17.1|

Mean values within rows with different superscripts are significantly different (P<0.05)
The ages at which 20 per cent or 80 per cent of the birds (progeny) would be susceptible for an intermediate IBD vaccine with a breakthrough titre of 250 were calculated using both commercial ELISAs (De Wit 1998, De Wit and Van Loon 1998, Block and others 2007) (Table 8).

### Table 2
BioChek infectious bursal disease (IBD) ELISA titres of breeders at different time points through lay

| BioChek ELISA | Vector IBD | Inactivated IBD |
|---------------|------------|-----------------|
| Age           | No.        | Mean            | 95% CI        | No.        | Mean            | 95% CI        |
| 15            | 10         | 4368a           | 3214 to 5555  | 10         | 4384a           | 3109 to 5660  |
| 22            | 10         | 6509a           | 5339 to 7680  | 10         | 8125b           | 6850 to 9401  |
| 27            | 19         | 7592a           | 6743 to 8441  | 21         | 6845a           | 5965 to 7726  |
| 32            | 20         | 5349a           | 4522 to 6177  | 20         | 5743b           | 4841 to 6645  |
| 36            | 20         | 5497a           | 4669 to 6324  | 20         | 6828b           | 5926 to 7730  |
| 41            | 20         | 5901a           | 5073 to 6728  | 20         | 6729a           | 5827 to 7631  |
| 45            | 18         | 6004a           | 5132 to 6877  | 19         | 6525b           | 5600 to 7451  |
| 56            | 20         | 7344a           | 6516 to 8172  | 19         | 7190b           | 6265 to 8116  |
| 60            | 19         | 6917a           | 6067 to 7766  | 20         | 7438a           | 6536 to 8340  |

Mean values within rows with different superscripts are significantly different (P<0.05)

### Table 3
IDEXX infectious bursal disease (IBD) ELISA titres of breeders at different time points through lay

| IDEXX ELISA titres | Vector IBD | Inactivated IBD |
|--------------------|------------|-----------------|
| Age                | No.        | Mean            | 95% CI        | No.        | Mean            | 95% CI        |
| 15                 | 10         | 4109a           | 2875 to 5135  | 10         | 4005a           | 2756 to 5254  |
| 22                 | 10         | 6033a           | 4903 to 7163  | 10         | 6051a           | 4802 to 7300  |
| 27                 | 19         | 5919a           | 5099 to 6739  | 20         | 5354a           | 4471 to 6237  |
| 32                 | 20         | 4596a           | 3797 to 5395  | 20         | 5211a           | 4328 to 6094  |
| 36                 | 20         | 5577a           | 4778 to 6376  | 19         | 6370a           | 5464 to 7276  |
| 41                 | 18         | 7598a           | 6756 to 8440  | 19         | 7800a           | 6894 to 8706  |
| 45                 | 18         | 6844a           | 6002 to 7668  | 19         | 7721a           | 6815 to 8627  |
| 56                 | 19         | 8189a           | 7369 to 9009  | 19         | 7456a           | 6550 to 8362  |
| 60                 | 17         | 7126a           | 6259 to 7993  | 17         | 10,173b         | 9215 to 11,130|

Mean values within rows with different superscripts are significantly different (P<0.05)

### Table 4
Virus neutralisation test of day-old progeny at different time points through the laying cycle

| Virus neutralisation test (log2) | Vector IBD | Inactivated IBD |
|---------------------------------|------------|-----------------|
| Age (weeks)                     | No.        | Mean            | 95% CI        | No.        | Mean            | 95% CI        |
| 32                              | 13         | 15.7a           | 14.9 to 16.5  | 19         | 16.0a           | 15.3 to 16.7  |
| 46                              | 18         | 15.7a           | 14.8 to 16.6  | 19         | 15.3a           | 14.4 to 16.2  |
| 61                              | 19         | 16.5a           | 16.0 to 17.1  | 20         | 16.3a           | 15.7 to 16.8  |

Mean values with different superscripts within rows or within columns are significantly different (P<0.05)

IBD, infectious bursal disease

### Table 5
BioChek ELISA titres of day-old progeny at different time points through the laying cycle

| BioChek ELISA titres | Vector IBD | Inactivated IBD |
|----------------------|------------|-----------------|
| Age (weeks)          | No.        | Mean            | 95% CI        | No.        | Mean            | 95% CI        |
| 32                   | 13         | 5686a1,2         | 4684 to 6687  | 19         | 6131a1          | 5302 to 6959  |
| 46                   | 18         | 4679a1           | 3693 to 5665  | 20         | 4279a1          | 3344 to 5215  |
| 61                   | 20         | 6299a2           | 5061 to 7537  | 20         | 5715a1          | 4477 to 6953  |

Mean values with different superscript letters within rows or with different superscript numbers within columns are significantly different (P<0.05)

IBD, infectious bursal disease

Parker D, de Wit S. Vet Rec Open 2014;1:e000016. doi:10.1136/vropen-2013-000016
DISCUSSION

The rearing birds had similar levels of humoral IBD antibodies as measured by virus neutralisation test and ELISA in both treatment groups, the antibody levels reported were uniform and in line with expectation. High levels of humoral antibodies to IBD were achieved at point of lay and persisted throughout the laying life of both groups. Both groups were housed on the same farm so if any IBD field challenge occurred during the laying period the impact could expect to be the same for both treatment groups. Humoral antibody levels in both treatment groups (as measured by both ELISA and VN) were numerically lower at the 32- and 36-week sampling than at the earlier and later sampling points. This relatively small drop in titre was observed around the period of production of peak egg mass. Naqi and others (1983) did not observe this drop in breeder IBD antibody level in their study; however, their first sampling point was two months into the laying period and the birds used in the Naqi study were White Leghorns rather than a commercial broiler breeder strain and known to respond differently to various vaccinal antigens. At 27 and 56 weeks, there was a significant difference in the humoral IBD antibodies as measured by virus neutralisation tests between the two treatment groups. Only at a single sampling point during the laying cycle was there a significant difference in the humoral IBD antibody level in the two treatment groups as measured by both ELISA tests. In this study, the IDEXX and BioChek IBD titres were in general comparable both in the breeders (Fig 1) and in the progeny (Fig 2). In previous work with conventionally vaccinated breeders (live IBD priming and inactivated vaccine), the BioChek IBD titres were significantly higher than the IDEXX IBD titres (De Wit and others 2001). Further studies are required to confirm whether this would be a consistent finding with the Vaxxitek HVT+IBD priming vaccination.

The humoral IBD antibody levels in the day-old progeny of these flocks did not show any significant difference between the treatment groups as measured by either SN (Fig 3) or ELISA (Fig 2) tests. Although mean levels of the IBD MDA were significantly different across the different sampling points as measured by both

### Table 6: IDEXX ELISA infectious bursal disease (IBD) antibody titres at different time points during the laying cycle

| IDEXX ELISA titres | Vector IBD | Inactivated IBD |
|--------------------|------------|-----------------|
| Age (weeks)        | No. | Mean | 95% CI | No. | Mean | 95% CI |
| 32                 | 13  | 4886 | 3789 to 5982 | 19  | 5614 | 4707 to 6522 |
| 46                 | 18  | 5585 | 4516 to 6654 | 19  | 4604 | 3564 to 5645 |
| 61                 | 20  | 7370 | 5892 to 8848 | 20  | 7111 | 5633 to 8590 |

Mean values with different superscript letters within rows or with different superscript numbers within columns are significantly different (P<0.05)

### Table 7: Virus neutralisation test and ELISA titres (BioChek and IDEXX) of breeders and their progeny

| GMT of breeders weeks 22–60 | GMT of progeny hatched at 32, 46 and 60 weeks | Percentage of transfer of antibodies to the progeny |
|-----------------------------|---------------------------------------------|-----------------------------------------------|
| Vector IBD                  | Inactivated IBD                            | Vector IBD (%) | Inactivated IBD (%) |
| VNT                         | 16.56                                      | 15.96 | 15.84 | 66 |
| BioChek                     | 12.46                                      | 12.14 | 12.07 | 80 |
| IDEXX                       | 12.55                                      | 12.34 | 12.29 | 86 |

IBD, Infectious bursal disease

### Table 8: The ages at which 20 per cent or 80 per cent of the birds (progeny) would be susceptible for a vaccine with a breakthrough titre of 250

| Age | Live vaccine | Inactivated vaccine |
|-----|--------------|---------------------|
|     | Age when 20% flock susceptible breakthrough titre | Age when 80% flock susceptible breakthrough titre |
|     | 250          | 250                 |
| Parent flock age | BioChek IDEXX | BioChek IDEXX | BioChek IDEXX | BioChek IDEXX |
| 32  | Too few samples | 16 | 15 | 19 | 19 |
| 46  | 14 | 14 | 18 | 18 | 14 | 14 | 18 | 17 |
| 61  | 16 | 16 | 19 | 20 | 12 | 14 | 19 | 20 |

Parker D, de Wit S. Vet Rec Open 2014;1:e000016. doi:10.1136/vropen-2013-000016

Open Access
ELISAs, when these titres were used to predicted vaccination dates using the Deventer formula the predicted dates for vaccination were not significantly different between the progeny of both treatment groups. This is an important observation for field veterinarians since day-old MDA as measured by ELISA is used in the field to help determine the optimal timing of live IBD vaccination in the broiler chicks (Lutticken and Cornelissen 1981, Block and others 2007). The level of transfer of antibodies from breeder to progeny (Table 7) was comparable to earlier results with these tests (De Wit and others 2001).

Vaxxitek HVT+IBD is a vectored vaccine using turkey herpes virus as the vector virus. PCR products of this
vaccine can be recovered from broiler breeder spleen and pulpy feathers late in the laying cycle (Sue Baigent, personal communication). If the Vaxxitek HVT+IBD vaccine can be detected in tissues of the avian immune system throughout the laying period of the broiler breeder, it can be assumed that the Vaxxitek HVT+IBD will continue to stimulate the immune system and maintain circulating IBD antibody levels in the breeder. This study confirms that humoral IBD antibody levels were maintained throughout the life of both treatment groups in these breeder flocks.

There was no significant improvement in the IBD humoral antibody response in the birds vaccinated with both Vaxxitek HVT+IBD and inactivated IBD vaccine compared with those vaccinated with Vaxxitek HVT+IBD alone. It is proposed that a single application of Vaxxitek HVT+IBD vaccine will confer the same level of protection to broiler breeders as a Vaxxitek HVT+IBD and inactivated vaccination programme and that these titres remain stable throughout the laying period.

Collaborators Helen Houghton Francesco Prandini.

Contributors Merial UK contributed to the trial by paying for the ELISA and SN testing at Deventer laboratory.

Competing interests None.

Provenance and peer review Not commissioned; externally peer reviewed.

Data sharing statement No additional data are available.

Open Access This is an Open Access article distributed in accordance with the Creative Commons Attribution Non Commercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited and the use is non-commercial. See: http://creativecommons.org/licenses/by-nc/4.0/

REFERENCES

Block H., Meyer-Block K., Rebeski D. E., Schar H., de Wit S., Rohn K., Rautenschlein S. (2007) A field study on the significance of vaccination against infectious bursal disease (IBDV) at the optimal time point in broiler flocks with maternally derived IBDV antibodies. Avian Pathology 36, 401–409

Bublot M., Pritchard N., Le Gros F. X., Goutorobe S. (2007) Use of a vectored vaccine against infectious bursal disease of chickens in the face of high-titred maternally derived antibody. Journal of Comparative Pathology 137(Suppl 1): S1–S8

Darteil R., Bublot M., Laplace E., Bouquet JF, Audonnet JC, Rivière M. (1995) Herpesvirus of turkey recombinant viruses expressing infectious bursal disease virus (IBDV) VP2 immunogen induce protection against an IBDV virulent challenge in chickens. Virology 211, 481–490

De Wit J. J. (1998) Gumboro disease: estimation of optimal time of vaccination by the Deventer formula. Polish Veterinary Journal 3, 19–22

De Wit J. J., Heijmants J. F., Mekkes D. R., Van Loon AA. (2001) Validation of five commercially available ELISAs for the detection of antibodies against infectious bursal disease virus (serotype 1). Avian Pathology 30, 543–549

De Wit J. J., Van Loon A. A. (1998) [Gumboro vaccination]. Tijdschrift voor diergeneeskunde 123, 7–10

Eidson C. S., Gelb J., Villegas P., Page R. K., Lukert P. D., Kleven S. H. (1980) Comparison of inactivated and live infectious bursal disease virus vaccines in White Leghorn breeder flock. Poultry Science 59, 2708–2716

Fahey K. J., Emly K., Crooks J. (1989) A conformational immunogen on VP-2 of infectious bursal disease virus that induces virus-neutralizing antibodies that passively protect chickens. The Journal of General Virology 70(Pt 6), 1473–1481

Flensburg M. F., Ernsboll A. K., Jorgensen P. H. (2002) Risk factors associated with the introduction of acute clinical infectious bursal disease among Danish broiler chickens in 1998. Avian Pathology 31, 23–29

Fussell L. W. (1998) Poultry industry strategies for control of immunosuppressive diseases. Poultry Science 77, 1193–1196

Lemiere S., Gauthier J., Kodjo A., Vinit L., Delvecchio A., Prandini F. (2013) Evaluation of the protection against infectious bursal disease challenge in progeny born to parents having received a vaccination program using herpesvirus of turkeys and infectious bursal disease (HVT-IBD) vector vaccine. World Journal of Vaccines 46–51

Lutticken D. (1997) Viral diseases of the immune system and strategies to control infectious bursal disease by vaccination. Acta veterina Hungarica 45, 239–249

Lutticken D., Cornelissen D. R. W. (1981) Plaquereducentiestand und Mitkronneutralisationstest zum Nachweis neutralisierender Antikörper gegen das Virus der infectiösen Bursitis (IBDV). Deutsche tierärztliche Wochenschrift 88, 506–508

Naqi S. A., Marquez B., Sahin N. (1983) Maternal antibody and its effect on infectious bursal disease immunization. Avian Diseases 27, 623–631

Sharma J. M., Kim I. J., Rautenschlein S., Yeh H. Y. (2000) Infectious bursal disease virus of chickens: pathogenesis and immunosuppression. Dev Comp Immunol 24, 223–235

Sharma J. M., Rautenschlein S., Sahin N. (1983) Maternal antibody and its effect on infectious bursal disease immunization. Avian Diseases 27, 623–631

Wochenschrift 88, 506–508

Van Den Berg T. P. (2000) Acute infectious bursal disease in poultry: a review. Avian Pathology 29, 175–194

Virology 70(Pt 6), 1473–1481

Poultry Science 77, 1193–1196

Acta Veterinaria Hungarica 45, 239–249

World Journal of Vaccines 46–51

Deutsche tierärztliche Wochenschrift 88, 506–508

Avian Diseases 27, 623–631

Deutsche tierärztliche Wochenschrift 88, 506–508

Deutsche tierärztliche Wochenschrift 88, 506–508