



# Comparative Evaluation of Live Vector, Immune Complex and Intermediate Plus Vaccines of Infectious Bursal Disease in Broiler Chicken

Rana Waqas Arshad<sup>1</sup>, Asim Aslam<sup>1</sup>, Muhammad Saeed Imran<sup>1</sup>, Kamran Ashraf<sup>2</sup> and Raheela Akhtar<sup>1,\*</sup>

<sup>1</sup>Department of Pathology, University of Veterinary and Animal Sciences, Lahore 54000

<sup>2</sup>Department of Parasitology, University of Veterinary and Animal Sciences, Lahore 54000

## ABSTRACT

The aim of the present study was to compare commercially available vaccines of infectious bursal disease (IBD). Three experimental groups, each having 100 broiler birds were placed in the same environmentally controlled house. Samples of blood and bursa of Fabricius were collected from slaughtered birds after every 7 days till the end of experiment (35 days). The size of bursas and the bursa/body weight ratios were significantly greater in live vector vaccine group than other vaccinal groups. ELISA revealed high antibody titre in live vector vaccine group and a partial protection was observed in birds vaccinated with immune complex or intermediate plus vaccines. Consistently, histopathological lesions of IBD were less evident in live vector vaccine group in comparison to other groups. In addition live vector vaccine improved the feed conversion ratio (FCR) by keeping the bird healthy and by decreasing the immunosuppression. These results indicated that live vector vaccine has overall positive impact in terms of immunity, histopathology of bursa of Fabricius and FCR. These results can be implemented in field for complete protection and better growth performance of broiler industry.

## Article Information

Received 25 May 2018

Revised 30 July 2018

Accepted 05 October 2018

Available online 05 July 2019

## Authors' Contribution

RWA and AA designed the study. RWA and MSI conducted the experiments. RA prepared the manuscript. KA and RA statistically analyzed the data.

## Key words

Antibody titre, Bursa weight, Feed conversion ratio, Infectious bursal disease.

## INTRODUCTION

Infectious bursal disease (IBD) is a great threat to poultry industry. It is highly contagious viral disease of young chicken (3-6 weeks of age) and is characterized by rapid onset, small duration and wide destruction of lymphocytes in bursa of Fabricius. Clinical signs include severe immunosuppression, trembling, prostration and whitish watery or mucoid diarrhea (Mekuriaw *et al.*, 2017).

The causative agent of IBD is a double stranded RNA virus. The stability of IBD virus to heat, ultraviolet radiation and photodynamic irradiation prolongs its survival in field (Michel and Jackwood, 2017). In addition the re-emergence of IBD virus in variant or highly virulent forms results in vaccine failure and significant economic losses (Soubies *et al.*, 2018). Therefore the reasonable control of IBD is only possible by quality vaccination. There have been many reasons for vaccine failure such as immunosuppression caused by IBD which not only decreases the response of infected chickens to IBD vaccine but to other vaccines such as Newcastle disease (ND), Marek's disease and infectious bronchitis (IB) as well.

As the vaccination is the primary method of IBD control in commercial poultry farms all over the world. Therefore, it is need of the time to use highly efficient, safe and secure IBD vaccine which may improve immunity and minimizes the pathological alterations in bursa (main target organ in this disease). In commercial poultry industry of Pakistan the birds are being immunized by different types of vaccines against IBD in which live vector vaccine, intermediate plus and immune complex are more common types. The objective of the present study was to compare these three commercially available vaccines for IBD to recommend the best one.

## MATERIALS AND METHODS

### Purchase of birds

Commercial broiler chicks (day old) were purchased from hatchery. Birds were housed in commercial broiler shed which was separated into three equal segments. Commercial rice husks provided as bedding were changed at two week intervals. Birds were kept in standard rearing conditions. Diet and clean water was offered *ad libitum*. Same practices were provided to all three treatment groups. A formal approval was obtained from ethical committee of University before conducting the experiments.

\* Corresponding author: [raheela.akhtar@uvas.edu.pk](mailto:raheela.akhtar@uvas.edu.pk)  
0030-9923/2019/0005-1837 \$ 9.00/0

Copyright 2019 Zoological Society of Pakistan

Table I.- Comparative growth performance and biometry of bursa and spleen after 1<sup>st</sup>, 2<sup>nd</sup>, 3<sup>rd</sup>, 4<sup>th</sup> and 5<sup>th</sup> week.

Vaccine	Body weight	House weight	FCR	Bursa weight (g)	Bursa size (mm)	Spleen weight (g)	Spleen size (mm)	Bursa/ Spleen ratio size	Bursa/ Spleen ratio weight	Bursa Body ratio
<b>Week 1</b>										
Vector	175.33±4.82 <sup>a</sup>	153.82±0.80 <sup>a</sup>	156.00±2.32 <sup>a</sup>	0.27±0.026 <sup>a</sup>	1.06±0.01 <sup>a</sup>	0.11±0.01 <sup>a</sup>	0.69±0.05 <sup>a</sup>	0.87±0.20 <sup>a</sup>	0.41±0.04 <sup>a</sup>	1.61±0.11 <sup>a</sup>
Immune complex	138.12±6.68 <sup>b</sup>	138.10±5.58 <sup>a</sup>	142.92±1.84 <sup>b</sup>	0.24±0.021 <sup>a</sup>	0.88±0.04 <sup>b</sup>	0.12±0.01 <sup>a</sup>	0.65±0.04 <sup>a</sup>	0.71±0.05 <sup>a</sup>	0.54±0.07 <sup>a</sup>	1.59±0.05 <sup>a</sup>
Intermediate plus	137.98±6.97 <sup>b</sup>	139.45±7.06 <sup>a</sup>	143.11±4.34 <sup>b</sup>	0.26±0.009 <sup>a</sup>	0.91±0.04 <sup>b</sup>	0.13±0.01 <sup>a</sup>	0.72±0.05 <sup>a</sup>	0.78±0.02 <sup>a</sup>	0.49±0.06 <sup>a</sup>	1.72±0.07 <sup>a</sup>
<b>Week 2</b>										
Vector	370.64±8.45 <sup>a</sup>	344.00±6.56 <sup>a</sup>	484.00±5.85 <sup>a</sup>	0.75±0.02 <sup>a</sup>	1.33±0.04 <sup>a</sup>	0.29±0.02 <sup>a</sup>	0.94±0.03 <sup>a</sup>	0.84±0.10 <sup>a</sup>	0.38±0.01 <sup>a</sup>	2.02±0.07 <sup>a</sup>
Immune complex	325.95±12.57 <sup>b</sup>	325.08±4.55 <sup>a,b</sup>	422.88±9.37 <sup>b</sup>	0.69±0.03 <sup>a</sup>	1.26±0.02 <sup>a,b</sup>	0.63±0.20 <sup>a</sup>	0.90±0.03 <sup>a</sup>	0.71±0.007 <sup>a</sup>	0.40±0.01 <sup>a</sup>	1.71±0.19 <sup>a</sup>
Intermediate plus	320.67±16.94 <sup>b</sup>	329.68±6.00 <sup>b</sup>	403.51±6.62 <sup>b</sup>	0.70±0.07 <sup>a</sup>	1.19±0.02 <sup>b</sup>	0.26±0.02 <sup>a</sup>	0.86±0.03 <sup>a</sup>	0.71±0.02 <sup>a</sup>	0.40±0.02 <sup>a</sup>	1.87±0.06 <sup>a</sup>
<b>Week 3</b>										
Vector	757.26±6.77 <sup>a</sup>	709.60±6.62 <sup>a</sup>	1018.80±5.45 <sup>a</sup>	1.47±0.09 <sup>a</sup>	1.74±0.11 <sup>a</sup>	0.65±0.06 <sup>a</sup>	1.25±0.05 <sup>a</sup>	1.02±0.29 <sup>a</sup>	0.46±0.02 <sup>a</sup>	2.12±0.05 <sup>a</sup>
Immune complex	710.06±6.19 <sup>b</sup>	664.40±9.02 <sup>b</sup>	950.24±24.96 <sup>b</sup>	1.26±0.17 <sup>a</sup>	1.56±0.11 <sup>a</sup>	0.67±0.58 <sup>a</sup>	1.22±0.05 <sup>a</sup>	0.78±0.02 <sup>a</sup>	0.55±0.03 <sup>a</sup>	1.96±0.06 <sup>a,b</sup>
Intermediate plus	682.76±11.38 <sup>c</sup>	655.80±7.55 <sup>b</sup>	917.31±22.65 <sup>a</sup>	1.20±0.11 <sup>a</sup>	1.45±0.02 <sup>a</sup>	0.67±0.14 <sup>a</sup>	1.19±0.03 <sup>a</sup>	0.81±0.03 <sup>a</sup>	0.58±0.05 <sup>a</sup>	1.78±0.10 <sup>b</sup>
<b>Week 4</b>										
Vector	1198.94±27.66 <sup>a</sup>	1092.60±12.15 <sup>a</sup>	1755.72±22.65 <sup>a</sup>	2.32±0.07 <sup>a</sup>	2.00±0.02 <sup>a</sup>	1.16±0.05 <sup>a</sup>	1.56±0.02 <sup>a</sup>	1.03±0.27 <sup>a</sup>	0.5±0.01 <sup>a</sup>	1.9±0.7 <sup>a</sup>
Immune complex	1070.01±39.61 <sup>b</sup>	1034.80±15.04 <sup>b</sup>	1564.79±41.29 <sup>b</sup>	1.75±0.21 <sup>a</sup>	1.84±0.10 <sup>a</sup>	1.29±0.14 <sup>a</sup>	1.59±0.05 <sup>a,b</sup>	0.83±0.06 <sup>a</sup>	0.5±0.06 <sup>a,b</sup>	1.5±0.2 <sup>a,b</sup>
Intermediate plus	1034.04±16.85 <sup>b</sup>	1045.00±17.60 <sup>b</sup>	1542.61±37.31 <sup>b</sup>	1.13±0.25 <sup>b</sup>	1.43±0.13 <sup>b</sup>	1.09±0.03 <sup>a</sup>	1.44±0.03 <sup>b</sup>	0.94±0.07 <sup>a</sup>	0.9±0.19 <sup>b</sup>	1.1±0.2 <sup>b</sup>
<b>Week 5</b>										
Vector	1653.00±14.47 <sup>a</sup>	1611.40±23.30 <sup>a</sup>	2599.20±91.65 <sup>a</sup>	2.39±0.12 <sup>a</sup>	2.22±0.08 <sup>a</sup>	2.12±0.16 <sup>a</sup>	1.97±0.02 <sup>a</sup>	0.92±0.06 <sup>a</sup>	0.88±0.16 <sup>a</sup>	1.47±0.23 <sup>a</sup>
Immune complex	1507.06±7.39 <sup>b</sup>	1566.80±23.19 <sup>a</sup>	2610.43±23.78 <sup>a</sup>	1.27±0.23 <sup>b</sup>	1.68±0.07 <sup>b</sup>	1.90±0.10 <sup>a,b</sup>	1.82±0.02 <sup>a,b</sup>	1.08±0.05 <sup>a</sup>	1.65±0.24 <sup>a,b</sup>	0.84±0.17 <sup>b</sup>
Intermediate plus	1408.23±36.82 <sup>c</sup>	1548.60±33.27 <sup>a</sup>	2518.23±50.49 <sup>a</sup>	0.98±0.09 <sup>b</sup>	1.38±0.08 <sup>c</sup>	1.56±0.10 <sup>b</sup>	1.75±0.08 <sup>b</sup>	1.04±0.09 <sup>a</sup>	1.16±0.12 <sup>b</sup>	0.79±0.13 <sup>b</sup>

### Experimental design

Three different groups (100 birds each) had different vaccination schedule at hatchery. The remaining management to all three groups including litter management, temperature, light management, ventilation, water and nutritional management was same.

The 1<sup>st</sup> and 2<sup>nd</sup> groups were injected with live vector vaccine (0.2mL/bird) and live immune complex vaccine (0.1mL/bird) of IBD subcutaneously and also coarse spray of live vaccine ND and IB at hatchery before delivering them to house. Birds in both groups were manually injected with killed oil based vaccine of ND 0.3mL/bird subcutaneously at 7<sup>th</sup> day of age, vaccines of live ND on day 11 and live IB on day 12. While for 3<sup>rd</sup> group schedule for the rest of vaccines was same except that live intermediate plus vaccine of IBD was given on 8<sup>th</sup> day of life.

### Sample collection

Bursal samples were collected on every 7<sup>th</sup> day of experiment after slaughtering 20 birds per group and gross lesions of bursa and spleen were recorded. Each sample was measured by ruler and weighed by electrical balance. Suitable samples were preserved in 10% neutral buffered formalin for histopathological examinations of these tissues as previously described (Mawgod *et al.*, 2014).

### Antibody titre

Blood samples (3-5mL) were collected from slaughtered birds and serum was separated to determine the ELISA titres (Mosley *et al.*, 2013)

### Statistical analysis

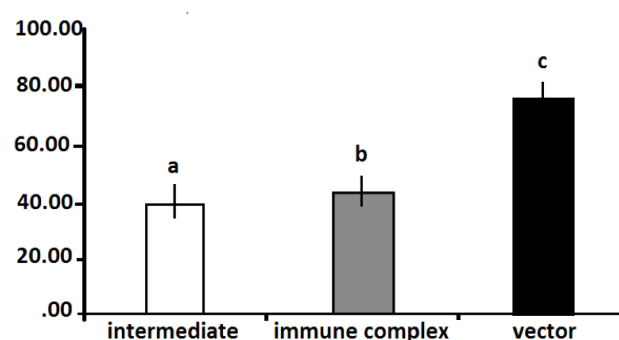
Data were presented in Mean  $\pm$  SEM and data on collection were analyzed statistically by analysis of variance (1 way ANOVA).

## RESULTS

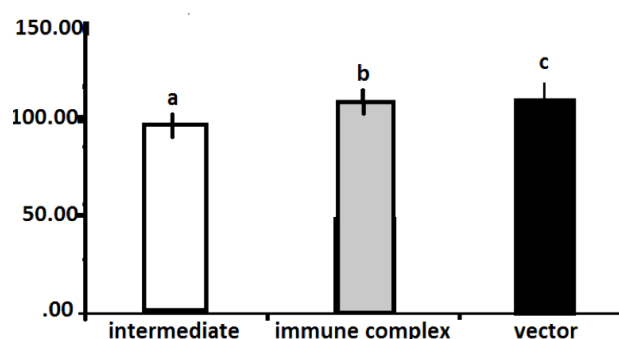
The present study was performed in order to compare the efficacy of live vector, immune complex and intermediate plus vaccine of IBD in commercial broilers. Our results indicated that live vector vaccine had positive impact on feed conversion ratio (FCR), antibody titre and histology of bursa as compare to immune complex and intermediate vaccine.

At 1<sup>st</sup> week of experiment there was a non significant difference in bursa weight and bursa to body weight ratio ( $P \geq 0.05$ ) in all three experimental groups. Moreover, all three groups had neither any histopathological alteration on bursa nor any significant gross change ( $P \geq 0.05$ ) in spleen sizes. However, live vector vaccine showed better FCR and significantly increased bursal size (Table I).

### ELISA Values of 3rd Week



### ELISA Values of 4th Week



### ELISA Values of 5th Week

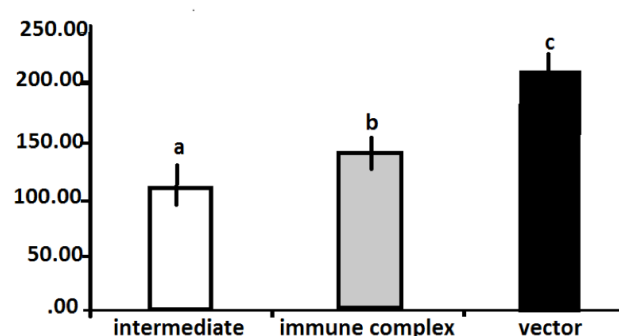


Fig. 1. ELISA values for live vector, immune complex and intermediate plus vaccines at 3<sup>rd</sup>, 4<sup>th</sup> and 5<sup>th</sup> weeks of age.

At 2<sup>nd</sup> week of experiment the live vector vaccine group had significantly better ( $P \geq 0.05$ ) FCR and bursal size as compare to immune complex and intermediate plus vaccines. While all three vaccines did not show any histopathological change in bursa and nor any significant change ( $P \geq 0.05$ ) in bursal and spleen weight (Table I). At 3<sup>rd</sup> week live vector vaccine showed better FCR as compare to other vaccinal groups but other parameters like bursa weight, bursa size, spleen weight, spleen size, bursa spleen size ratio, bursa spleen weight ratio and bursa body weight

ratio were non significantly different ( $P \geq 0.05$ ) (Table I). Moreover at 3<sup>rd</sup> week of experiment the live vector vaccine showed significantly higher antibody titre as compare to two other vaccines.

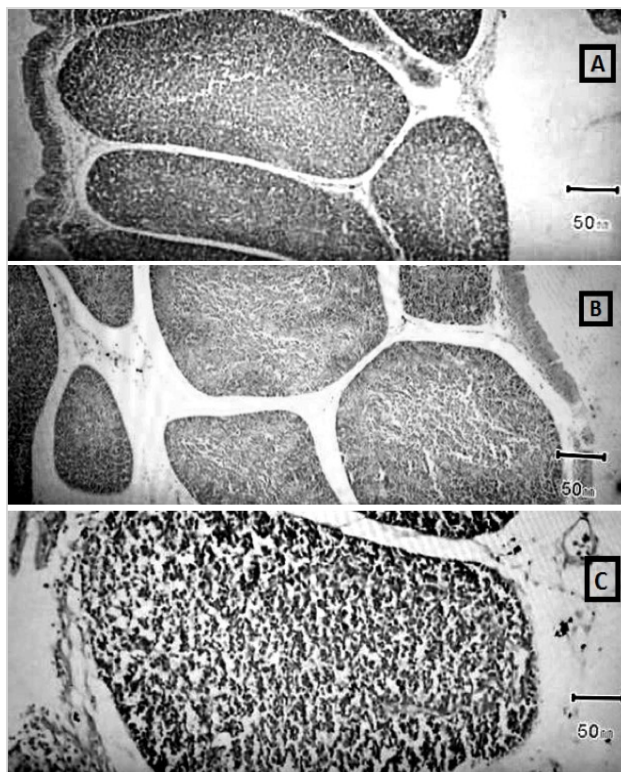


Fig. 2. Normal histology of follicles, cortex and medulla seen in bursa from vector vaccine (A) group. While immune complex (B) and intermediate plus (C) vaccine groups showed depletion of lymphocytes in medullary region.

At 4<sup>th</sup> week FCR and antibody titre of live vector vaccine was significantly ( $P \geq 0.05$ ) improved than other two groups. The bursa weight, size and bursa body weight ratio were not significantly different ( $P \geq 0.05$ ) in vector and immune complex but significantly different ( $P \geq 0.05$ ) in intermediate plus group (Table I). At 5<sup>th</sup> week FCR was significantly better ( $P \geq 0.05$ ) in live vector and immune complex vaccine than intermediate plus vaccine. However, the bursa weight and size and antibody titre was significantly better ( $P \geq 0.05$ ) in vector vaccine than immune complex and intermediate plus vaccine (Fig. 1). Spleen weight and size was significantly improved ( $P \geq 0.05$ ) in vector vaccine and significantly different ( $P \geq 0.05$ ) in immune complex and intermediate plus (Table I). Our results showed that bursa samples from live vector vaccine group had normal histology of follicles and distinct cortex and medulla

were observed microscopically (Fig. 2A). While the birds immunized with immune complex vaccine showed few changes in the lymphoid follicles and lymphocytes were depleted in the germinal centres of the follicles. In addition most of the central areas were occupied by stroma (Fig. 2B). Similarly in intermediate plus vaccine group, the bursal samples showed mild changes and some depletion of cells in the medullary region (Fig. 2C).

## DISCUSSION

This is the first comprehensive report on comparison of live vector, immune complex and intermediate plus vaccines of infectious bursal disease on integrity of bursa of Fabricius and performance of commercial broiler.

Our results indicated that the live vector vaccine can significantly protect the bursal health with measurable results on humoral immune system. This is in agreement with previous studies of Ismail and Saif (1991) who described that live IBD vaccines are highly efficient in controlling the disease. This may be due to the nature of live vector vaccine that is a genetically modified vaccine in which only single gene (VP2) is carried by a vector. It may also be due to the ability of live vaccine to overcome maternally derived antibodies as these antibodies may decline the efficacy of live vaccine (Tsukamoto *et al.*, 2002; Bublot *et al.*, 2007).

The results of present study revealed that the body weight of birds immunized with live vector vaccine were significantly higher ( $P \leq 0.05$ ) than the birds in other vaccine groups. This may be due to stress associated with intermediate plus vaccines due to double administration. The stress affected the growth performance and decreased feed intake which ultimately reduced the body weight.

Another method for evaluation of immunity is the assessment of lymphoid organ's weight in poultry. The evaluation of bursal size, weight and bursal index (BI) (bursa weight: body weight ratio) is the most commonly caused model to estimate protection rate given by vaccines against IBD (Bolis *et al.*, 2003) and biological measure of overall health status. Sick or stressed birds have small bursa while healthy protective birds have large bursa (Yegani and Korver, 2008). As the live vector vaccine kept the bursa healthy and bursa weight and size were significantly better ( $P \leq 0.05$ ) at 5<sup>th</sup> week of age as compared to other vaccines used it indicated the better efficacy of live vector vaccine. These results were also verified by estimating antibody titre in ELISA.

Normally the bursa to body weight ratio increases in first five weeks due to strong bursal development as compared to body development. But from 6<sup>th</sup> week onwards the bursa to body weight (BBW) ratio decreases due to

stabilization of bursal development. In present study, the bursal growth was consistent ( $P \leq 0.05$ ) up to 5 weeks in group immunized with live vector vaccine while the other two groups BW ratio was reduced due to destruction of bursal integrity in 5<sup>th</sup> Week.

Damage to the bursa by IBD virus leads to immunosuppression and lesion development (Hoerr, 2010). Histopathological examination revealed normal lymphoid follicles and distinct cortex and medulla in bursa of live vector vaccine group. While in case of immune complex group, there were some mild changes in follicles along with some depletion of lymphocytes. On other hand birds vaccinated with intermediate plus vaccine had mild to severe changes with depletion of immune cells in the medullary region and follicles.

On the basis of these results we concluded that comparatively better protection, better growth performance and least pathological lesions were observed by using live vector vaccine in broiler chicken as compare to immune complex and intermediate plus vaccine. Therefore, its use is recommended in broiler chickens against IBD.

#### ACKNOWLEDGEMENT

The present study was funded by Department of Pathology, University of Veterinary and Animal Sciences, Lahore, Pakistan.

#### Statement of conflict of interest

The authors declare that there is no conflict of interest regarding publication of this article

#### REFERENCES

- Bolis, D.A., Paganini, F.J., Simon, V.A., Zuanaze, M., Scanavini, N.H. and Correa, A., 2003. Gumboro disease: Evaluation of serological and anatomopathological response vaccinated broiler chicken challenged with very virulent virus strain. *Braz. J. Poult. Sci.*, **5**: 137-146. <https://doi.org/10.1590/S1516-635X2003000200008>
- Bublot, M., Pritchard, N., Le-Gros, F.X. and Goutebroze, S., 2007. Use of a vectored vaccine against infectious bursal disease of chickens in the face of high-titred maternally derived antibody. *J. Comp. Pathol.*, **137**: 581-584. <https://doi.org/10.1016/j.jcpa.2007.04.017>
- Hoerr, F.J., 2010. Clinical aspects of immunosuppression in poultry. *Avian Dis.*, **54**: 2-15. <https://doi.org/10.1637/8909-043009-Review.1>
- Ismail, N.M. and Saif, Y.M., 1991. Immunogenicity of infectious bursal disease viruses in chickens. *Avian Dis.*, **35**: 460-469. <https://doi.org/10.2307/1591208>
- Mawgod, S.A., Arafa, A.S. and Hussein, H.A., 2014. Molecular genotyping of the infectious bursal disease virus (IBDV) isolated from broiler flocks in Egypt. *Int. J. Vet. Sci. Med.*, **2**: 46-52. <https://doi.org/10.1016/j.ijvsm.2014.02.004>
- Mekuriaw, A., Bitew, M., Gelaye, E., Mamo, B. and Ayelet, G., 2017. Infectious bursal disease: Outbreak investigation, molecular characterization, and vaccine immunogenicity trial in Ethiopia. *Trop. Anim. Hlth. Prod.*, **49**: 1295-1302. <https://doi.org/10.1007/s11250-017-1328-2>
- Michel, L.O. and Jackwood, D.J., 2017. Classification of infectious bursal disease virus into genogroups. *Arch. Virol.*, **162**: 3661-3670. <https://doi.org/10.1007/s00705-017-3500-4>
- Mosley, Y.C., Wu, C.C. and Lin, T.L., 2013. Infectious bursal disease virus rescued efficiently with 3' authentic RNA sequence induces humoral immunity without bursal atrophy. *Vaccine*, **31**: 704-710. <https://doi.org/10.1016/j.vaccine.2012.11.040>
- Soubies, S.M., Courtillona, C., Abedb, M., Amelota, M., Keitaa, A., Broadbentc, A., Härtled, S., Kaspersd, B. and Etteradossia, N., 2018. *Avian Pathol.*, **47**: 179-188. <https://doi.org/10.1080/03079457.2017.1393044>
- Tsukamoto, S., Saito, S., Saeki, S., Sato, T., Tanimura, N., Isobe, T., Mase, M., Imada, T., Yuasa, N. and Yamaguchi, S., 2002. Complete, long-lasting protection against lethal infectious bursal disease virus challenge by a single vaccination with an avian herpesvirus vector expressing VP2 antigens. *J. Virol.*, **76**: 5637-5645. <https://doi.org/10.1128/JVI.76.11.5637-5645.2002>
- Yegani, M. and Korver, D.R., 2008. Factors affecting intestinal health in poultry. *Poult. Sci.*, **87**: 2052-2063. <https://doi.org/10.3382/ps.2008-00091>