XXXI Ciclo di Dottorato in Scienze Veterinarie Dott. Flavio Silveira
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Dynamic and tissue distribution of an Italian variant of Infectious Bursal Disease Virus in SPF chickens.

INTRODUCTION

Infectious bursal disease (IBD) is a worldwide, highly contagious disease of chickens caused by an Avibirnavirus named IBDV (Van Den Berg et al., 2000). IBDV has a bi-segmented double-stranded RNA genome and during its replicative cycle destroys the developing B-lymphocytes in the bursa of Fabricius, resulting in immunosuppression and relevant economic due to the increased susceptibility to secondary infections and sub-optimal response to vaccinations (Balamurugan and Kataria, 2006).

An emerging IBDV genotype (ITA) was detected in Italy in IBDV-live vaccinated broilers without IBD clinical signs. VP2 sequence analysis of field isolates of ITA showed that strains of the this genotype clustered separately from other IBDV reference strains, either classical or very virulent, retrieved from GenBank or previously reported in Italy, and from vaccine strains. It seems to be emergent in Italian densely populated poultry areas being the 68 % of the IBDV detections made during routine diagnostic activity over a two-year period (2013-2014) (Lupini et al., 2016).

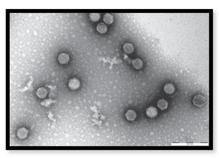
AIM

To deepen the pathological characteristics of an IBDV ITA genotype strain through the study of the persistence and tissue distribution in SPF chickens.

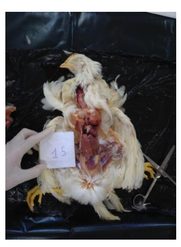
MATERIALS AND METHODS

1. Experimental design

45 one-day-old Specific Pathogen Free (SPF) chickens were used in the trial. The chickens were divided into 2 groups: infected group (ITA), composed by 30 birds and a negative control group including 15 birds. The groups were kept in two separate poultry isolators.



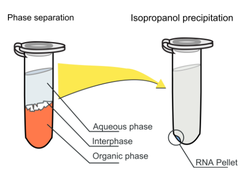
At 35 days of life each birds of group ITA were inoculated via oral route with a viral dose of 10^4 of the field isolate IBDV/Italy/1829/2011. The control group was mock-inoculated.



Five animals from group ITA and three animals from the control group were euthanized in the following days post-infection (d.p.i.): 2, 4, 7, 14, 21 and 28. From each euthanized bird cloacal swabs and tissue samples from bursa of Fabricius, kidney, liver, spleen, caecal tonsils, thymus, Harder gland and bone marrow were collected.

2. RNA extraction

Tissues and swabs were homogenates in phosphate buffer solution (PBS) and supernatants were mixed with guanidinium isothiocyanate and denaturation solution in sterile 2 ml tubes. These mixtures were freeze-thawed for 3 h before of the treatment with sodium acetate and phenol-chloroform-isoamyl alcohol.



The RNA from the aqueous phase was precipitated by the addition of an equal volume of isopropanol and stored overnight. Then the substances were pelleted by centrifugation and The RNA was air-dried and re-dissolved water.

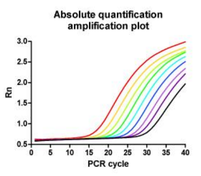
3. Real Time RT-PCR for IBDV

A specific one-step Real Time RT-PCR (qRT-PCR) protocol was developed and performed for this study. Strain-specific primers and probe are described in Table I.

Table I. Primers and probes used for qRT-PCR of IBDV genomes.

Primer	ITA
Forward	CTCAGCCTGCCCACATCATA
Reverse	CGTTACCCCACCTTGTTGGT
Probe	AGGCTTGGWGACCCCATTCC

The reaction used were: 50 ºC for 15 min of RT step, 95 ºC for 2 min, and 45 cycles of 95 ºC for 15 sec, and 60 ºC for 30 sec for the PCR step. All samples were run in duplicates with two positive controls and a negative control. Data were analyzed using LightCycler® Nano Software V1.1.



A standard curve was generated using cycle threshold (Ct) values obtained from qRT-PCR carried out on RNAs of the titrated IBDV/Italy/1829/2011 on 10-fold serial dilution. The viral RNA (vRNA) was quantified by comparison with this standard curve.

RESULTS

1. Distribution and persistence of IBDV/Italy/1829/2011 viral RNA in tissues

Table II. Detection of IBDV in lymphoid and non-lymphoid tissues.

d.p.i.	lymphoid tissues						non-lymphoid tissues			
	Bursa of fabricius	spleen	thymus	bone marrow	caecal tonsils	harder gland	kidney	liver	proventriculus	Cloacal swabs
2	5/5[a]	5/5	5/5	5/5	4/5	3/5	2/5	5/5	0/5	4/5
4	5/5	3/5	5/5	5/5	5/5	1/5	4/5	4/5	1/5	0/5
7	5/5	5/5	5/5	5/5	2/5	2/5	5/5	3/5	1/5	1/5
14	5/5	4/5	5/5	5/5	4/5	1/5	4/5	1/5	0/5	0/5
21	5/5	3/5	0/5	2/5	5/5	1/5	1/5	0/5	1/5	0/5
28	4/5	1/5	2/5	4/5	5/5	3/5	0/5	0/5	0/5	0/5

[a]IBDV positive /total sampled.

- All lymphoid tissues were positive for IBDV up to the end of the trial.
- Non-lymphoid tissues were positive up to 21 d.pi.
- Cloacal swabs a clearance point was observed at 4 d.p.i

2. Quantification of the vRNA in lymphoid and non-lymphoid tissues

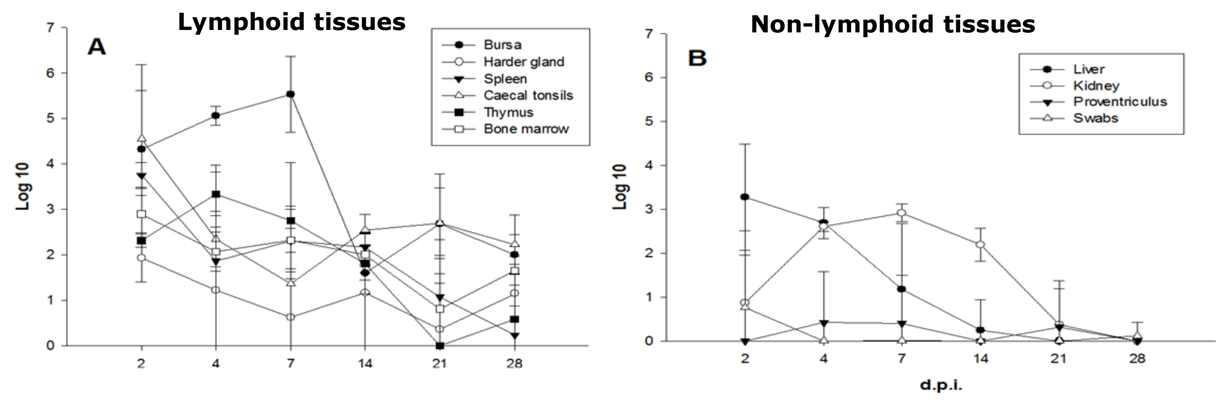


Figure 1. Viral load of IBDV in lymphoid and non-lymphoid tissues expressed in Log_{10} with 95% of confidence interval. A) Lymphoid tissues inoculated with ITA; B) Non-lymphoid tissues inoculated with ITA.

DISCUSSION AND CONCLUSION

This study shows that ITA vRNA can be detected in experimental conditions in SPF chickens for up to 28 d.p.i. in lymphoid organs, especially in bursa of Fabricius, caecal tonsil and bone marrow.

Interestingly, similar loads of vRNA in these organs were found in the end of the experiment. Hence, it indicates that caecal tonsils and bone marrow may serve as non-bursal lymphoid tissues that support virus persistence in chickens at later time points post-infection.

Publications

•Silveira F., Maluta R.P., Tiba M.R., De Paiva J.B., Guastalli E.A.L., Da Silveira W.D. (2016) "Comparison between avian pathogenic (APEC) and avian faecal (AFEC) Escherichia coli isolated from different regions in Brazil". Vet. Jou., 2016 in press. doi:10.1016/j.tvjl.2016.06.007

Oral communication and posters

•Lupini C., Silveira F., Felice V., Berto G., Meini A., Franzo G., Cecchinato M., Mercolini G., Listorti V., Catelli E. (2016). "Patogenicità del virus della bursite infettiva genotipo ita in polli SPF". Atti della Società Italiana di Patologia Aviare 2016., pag.187.
•Felice V., Franzo G., Catelli E., Bonci M., Cecchinato M., Giovanardi D., Pesente P., Mescolini G., Listorti V., Silveira F., Lupini C. (2016). "Analisi della sequenza completa del virus della bursite infettiva genotipo ITA". Atti della Società Italiana di Patologia Aviare 2016., pag.157.

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•Lupini, C., Giovanardi, D., Pesente, P., Bonci, M., Felice, V., Rossi, G., Morandini, E., Cecchinato, M., Catelli, E., 2016. A molecular epidemiology study based on VP2 gene sequences reveals that a new genotype of infectious bursal disease virus is dominantly prevalent in Italy. Avian Pathol. 45, 458–464. doi:10.1080/03079457.2016.1165792
•Van Den Berg, T.P., Eterradossi, N., Toquin, D., Meulemans, G., 2000. Infectious bursal disease (Gumboro disease). OIE Rev. Sci. Tech. 19, 527–543.